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# STUDIES IN EXPERIMENTAL GLYCOSURIA.

## XI. Retention of Dextrose by the Liver and Muscles and the Influence of Acids and Alkalies on the Dextrose Concentration of the Blood

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A considerable amount of evidence has been accumulating in recent years to indicate that the behavior of carbohydrate in the animal body is in part dependent upon the reaction of the tissue fluids. Since conditions such as dyspnoea, etc., which are known to raise the H-ion concentration of the body fluids, cause hyperglycaemia and glycosuria, it was suggested by one of us several years ago that such an increase in the H-ion concentration of the blood supplying the liver cells might stimulate the glycogenolytic process. Direct evidence in favor of this view—and also showing that the opposite change in reaction (increased alkalinity) has a glycogen-forming influence (at least in the turtle liver)—was furnished by Elias (1), who found in rabbits, after administration of dextrose along with acid, that distinctly less glycogen accumulated in the liver and that the blood-sugar rose much more than after dextrose alone. Glycosuria also became established in the former but not in the latter case. Similar results were obtained by this investigator in dogs; thus, the administration of an amount of dextrose which ordinarily caused no glycosuria did cause it when the dextrose was given along with mineral acid, the blood-sugar being also considerably raised in the latter cases. The amounts of acid used in these experiments were such as had previously been shown by Walter to produce distinct changes in the reaction of the blood. Elias and

Kolb (2) subsequently showed that starvation diabetes in dogs is accompanied by acidosis, and that the hyperglycaemia produced by giving a certain amount of dextrose to such animals is more pronounced than that observed after the same amount of dextrose given to normal animals.

Regarding the action of alkali in causing a decrease in the sugar-concentration of the blood, or, in cases of glycosuria, in that of the urine, several researches have recently been recorded. These date from the discovery, announced in 1911 by Pavy and Bywaters (4), that injection of alkali into the portal system inhibits post-mortem glycogenolysis in the liver, and that the glycosuria usually produced by ether and chloroform in cats is abolished by the injection intravenously of about 0.75 grams per kilogram of sodium carbonate (Pavy and Godden (5)). These authors also stated, though they gave no data, that irritation of the vagus nerve in the neck failed to cause glycosuria in cats to which sodium carbonate had previously been given. Murlin and Kramer (6) have discovered that the glycosuria in complete pancreatic diabetes can be caused to disappear by the injection of 300 cc. of a 1 per cent solution of $Na_2CO_3$, and that meanwhile the blood-sugar does not at least increase, and Underhill (7) has recently shown in normal rabbits that the intravenous injection of 80 to 100 cc. of 0.4 per cent $Na_2CO_3$ causes a hypoglycaemia lasting for one to one and one-half hours, but capable of being maintained by renewal of the alkaline injection. By administration of alkali (0.5 grams) at an interval of one-half to one hour previous to that of adrenin, this author has also found that the blood-sugar does not rise so high, nor does the increase last for so long a time, as in normal rabbits after a similar injection of adrenin, and the glycosuria is much less.

The undoubted hypoglycaemic effect of alkali administration and the probable hyperglycaemic effect of acid might be explained as due either to an influence on the glycogenolytic process in the liver (and muscles) or to alterations in the rate of sugar utilization of the tissues. To throw light on this question, Murlin and Kramer (6) have observed the behavior of the respiratory quotient following the administration of $Na_2CO_3$ to normal, and to partially and totally depancreated, dogs. No increase in the respiratory quotient was found to occur in the normal animal, but when the alkali was given intravenously, along with dextrose to a diabetic animal, whose urine had been rendered sugar-free even while on full diet, by previous administration of $Na_2CO_3$, a distinct rise in the respiratory quotient was observed. A limited capacity

to oxidise glucose, in another partially depancreated dog, was found to be increased by giving $Na_2CO_3$ along with glucose, although $Na_2CO_3$ alone had no influence. Administration of the carbonate and glucose was also found by these authors to cause a marked rise in the respiratory quotient on the fifth day after total pancreatectomy in a dog.

These results are interpreted as indicating that the alkali stimulates increased utilization of dextrose in the tissues. This might alone be sufficient to account for the decrease in blood-sugar concentration, but there is nothing in the experiments which negatives the view, originally suggested by Pavy's work above referred to, that an equally important cause may be a lessening in the rate of discharge of dextrose from the liver. To investigate this possibility, Kramer and Marker (8) estimated the glycogen in the liver and muscles of a totally depancreated dog that had been caused to retain considerable quantities of dextrose while on a meat diet, by administration of sodium carbonate. Since only traces were found, they conclude that the "retained" dextrose cannot have been stored as glycogen. Evidently recognizing the possible fallacies that are well-known to be incurred in such experiments, the authors performed a second experiment, in which a totally depancreated dog was first of all rendered a-glycogenic by adrenin administration, and was then given meat and carbonate until considerable glucose, which must have been derived from protein, had been retained. On again giving adrenin, it was found that no increase in sugar-excretion occurred, thus indicating that "none of the glucose retained had been stored as glycogen," nor could the dextrose be shown to be "eliminated by way of the saliva or the gastro-intestinal tract." Until further details are available it is impossible to appraise the value of the results of these well-conceived experiments.

The apparent rapidity with which the hypoglycaemic effect of alkali becomes developed (cf. Underhill) suggested to us that it might be possible to decide whether this depends on a depression of the glycogenolytic activity of the liver, by comparison of the sugar-content of the blood flowing into and out of the liver, and likewise whether there was increased utilization (or storage) of dextrose by the muscles by watching the behavior of the sugar in the venous blood of muscles. This has been the general nature of the experiments herein recorded, and in undertaking them we have recognized the fact that it is only when a very considerable retention or secretion of the sugar is occurring that any changes in the comparative amounts of sugar in the inflowing and outflowing blood are likely to be encountered. This objection

however applies mainly when the attempt is made to base conclusions on the analysis of one or two samples of blood removed simultaneously from the two vessels. When the samples are removed from the two vessels at frequent intervals, thus permitting curves to be drawn, the method becomes of much greater value, although the differences can never be large. The introduction of methods for rapid sugar estimation in small amounts of blood (2 cc.) has rendered such investigations possible.

Besides the above mentioned investigations concerning the influence of acid and alkali on the behavior of the blood-sugar, we have incidentally collected some data bearing on the cause of the very rapid disappearance of intravenously injected dextrose. A very complete review of the literature bearing on this subject has recently been published by Kleiner (9). The main fact is that, after the injection of large amounts of dextrose, for example about 4 grams per kilo body weight, the injected sugar rapidly disappears from the blood (in ninety minutes in Kleiner's experiments). Although under ordinary conditions some of this excess of dextrose escapes with the urine, it was found by Kleiner still to disappear at about the same rate in animals from which the kidneys had been removed. In Kleiner's experiments conversion into glycogen by the liver could not be held accountable for the disappearance of the dextrose, since a similar disappearance also occurred in animals having no circulation posterior to the level of the diaphragm. It was found that the muscular tissues absorb the excess of sugar possibly by building some of it up into polysaccharides.

There is one serious objection to all of the experiments of the above type, namely, that by the rapid addition of excessively large quantities of dextrose to the blood, conditions are established which can never obtain in even the severest varieties of experimental diabetes. Apart from the disturbance of osmotic relationships between the blood and tissue fluid created by such injections, the excess of dextrose, either because of its own presence or because of decomposition products derived from it (e.g., lactic acid, etc.), may completely throw out of working order those mechanisms which under ordinary circumstances would take care of any physiological excess of dextrose.

Notwithstanding these criticisms, the experiments of Kleiner and others are noteworthy in that they show the glycogenic function of the liver to be of practically no consequence in absorbing the excess of dextrose. This conclusion prompted us in the present investigation to make some observations on the relative sugar-retaining power of the

liver and muscles during the continuous injection into the portal vein of large quantities of dextrose.

In the majority of our experiments we have employed for injection quantities of dextrose that were below the normal tolerance limit as defined by Woodyatt, Sansum and Wilder (13). These authors have shown that, in a normal rabbit, dog or man, 0.9 gram glucose per kilo body weight and per hour can be utilized by the organism for an indefinite time without causing glycosuria. When between 0.8 and 2 grams are injected a part of the excess appears in the urine, steadily increasing until a maximum is reached, after which the excreted fraction remains constant (at about one tenth). If more than about 2 grams per kilogram an hour are injected, "a large percentage of all glucose in excess of the 2 grams per kilogram an hour appears in the urine once constant conditions are established."

In the light of these observations we should expect that the curve of blood-sugar concentration during continuous injections of dextrose would gradually rise to a certain level, after which it would remain constant at a height proportional to the rate of injection. With injections below the tolerance limit, the rate of injection at which the curve ceases to rise would indicate the rate at which the tissues are consuming the dextrose, and it is when this point of the curve is reached that the influence of various conditions on sugar utilization by the tissues could be most simply studied. When larger amounts of dextrose are given, the level would represent the balance between sugar loss by the urine *plus* its utilization by the tissues, on the one hand, and injection rate, on the other.

*Technique.* 2 cc. samples of blood were collected, in the manner previously described (10), from the pancreatico-duodenal vein, the inferior vena cava opposite the liver, and in some experiments also, from the common iliac vein, after ligation of the vessels of the opposite posterior extremity. The samples were collected before, during and following the injection, through a cannula inserted in one of the smaller tributary branches of the portal vein, of the solution whose influence on blood-sugar concentration it was desired to study. To maintain a constant rate of injection—which is absolutely essential in all work designed to study the fate of injected dextrose—either the injection apparatus described in a previous paper or Woodyatt's apparatus (13) was used. Unfortunately, after some of the earlier experiments had been completed, it was found that the air pressure used in the former apparatus was insufficient to ensure a perfectly uniform rate of injection, with the consequence that some of the results are confusing and un-

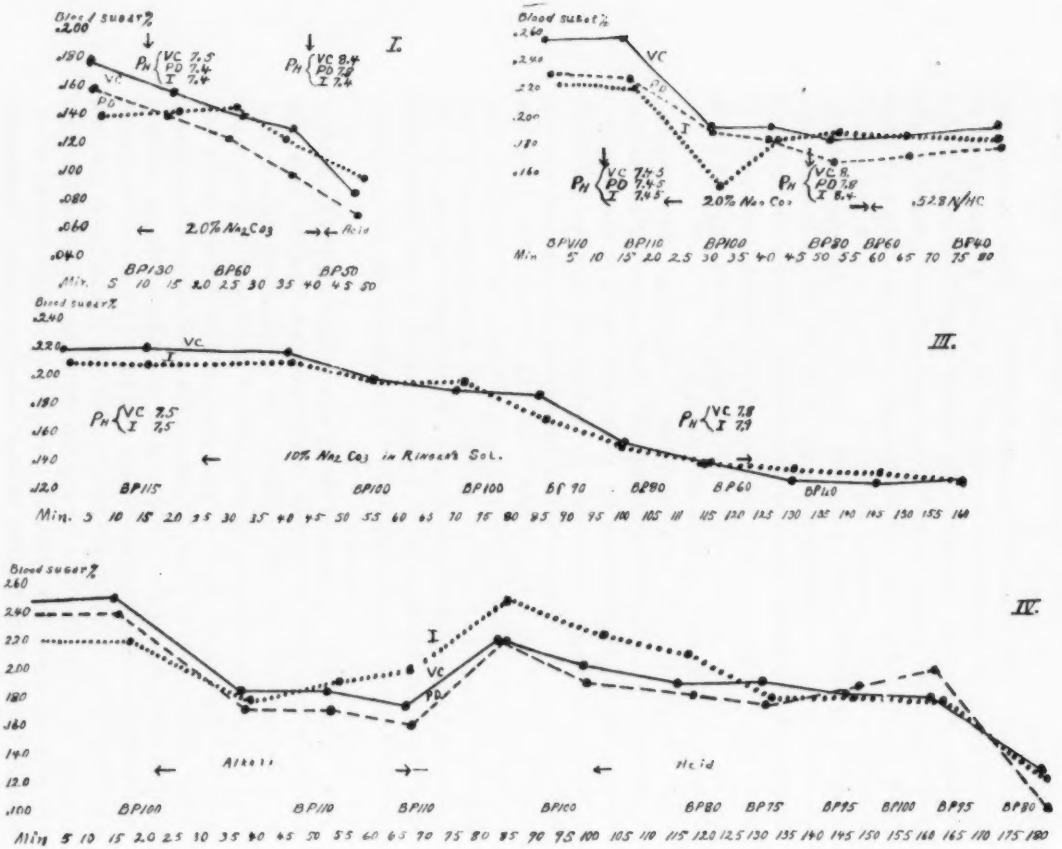
I.
II.
III.
IV.
Blood sugar %
Min.
2.0% Na2CO3
Acid
0.528 N/HC
10% Na2 Co3 in Ringer's Sol.
Alkali
VC
PD
I

Fig. 1.

certain. Woodyatt's apparatus is, in our experience, unqualifiedly the best to use.

The sugar estimations were made by R. G. Pearce's modification of the Lewis-Benedict method (11), care being taken that the temperature conditions in the autoclave were the same for all the blood samples in the given experiment. This eliminates any error, in so far as comparative results are concerned, that might be incurred by an unequal degree of heating of different tubes.

Small samples of blood (about 5 cc. each) were also removed at intervals for the determination of the H-ion concentration by the dialysis colorimetric method of Levy, Rowntree and Marriott (12).

## EXPERIMENTAL RESULTS

*1. The hypoglycaemic influence of alkali.* To study the hypoglycaemic influence of alkali, a strong solution of $Na_2CO_3$ was injected at constant rate into a small branch of the mesenteric vein of dogs to which, on the evening previous to the experiment, about 6 grams per kilogram of cane sugar had been administered by stomach tube. The results of these experiments are plotted in the four curves shown in figure 1.

Fig. 1. The influence of large injections of sodium carbonate on the sugar-content of blood from the pancreatico-duodenal vein, vena cava, and iliac vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I.*, iliac, dotted line; $P_H$, H-ion concentration; *B.P.*, arterial blood-pressure. The injections were made between the points of the arrows.

*Experiment I.* Sugar fed dog. Weight, 7.5 kg. Kennel fed plus 50 grams of cane sugar, given by stomach tube 20 hours before the collection of blood. Air pressure injection apparatus used, set to inject 50 cc. per hour. Solutions injected: (1) 20 per cent $Na_2CO_3$; (2) 0.346 N/HCl.

*Experiment II.* Sugar fed dog. Weight, 8.8 kg. Kennel fed plus 50 grams of cane sugar given by stomach tube 18 hours before collection of blood. Used Woodyatt's perfusion apparatus. Solutions injected: (1) 20 per cent solution of $Na_2CO_3$; (2) 0.528 N/HCl solution. Both solutions were injected at the rate of 1 cc. per minute.

*Experiment III.* Sugar fed dog. Weight, 7.8 kg. Kennel fed plus 46 grams of cane sugar, given by stomach tube, on each of two days previous to that of the experiment. Used Woodyatt's perfusion injecting apparatus. Solutions injected: (1) 10 per cent $Na_2CO_3$ and 0.528 N/HCl. Both solutions were injected at the rate of 1 cc. per minute.

*Experiment IV.* Sugar fed dog. Weight, 21 kg. Kennel fed plus 100 grams of cane sugar, given by stomach tube 20 hours before the collection of blood. Used Woodyatt's perfusion injection apparatus. Solutions injected: (1) 10 per cent $Na_2CO_3$; (2) 0.528 N/HCl. Both solutions were injected at the rate of 1 cc. per minute.

In experiment I the alkaline solution (20 per cent $Na_2CO_3$) was injected rapidly (considerably more than 1 cc. a minute). In II the same strength of alkali was more slowly injected (1 cc. a minute). In III and IV a 10 per cent solution of $Na_2CO_3$ was employed, the blood pressure being well maintained in IV, but falling fairly rapidly in III.

In Experiments I, II, III, the H-ion concentration of the blood, indicated by the figures standing opposite PH, was very considerably lowered by the injections, and this was no doubt also the case in IV, although PH was not determined in this experiment. In the first two experiments the alkali had a marked depressing influence on the animal (as indicated by the behavior of the arterial blood pressure), death occurring in one of them after about forty minutes. This depression was less marked in animals 3 and 4. The addition to the blood of such large quantities of alkali as were employed in these experiments is obviously far in excess of what could be tolerated therapeutically. Such quantities were used in order to ascertain to what extent a marked increase in the alkalinity of the blood and tissues could reduce the sugar-concentration of the blood.

A steady decrease in blood sugar is evident in all the experiments. This decrease might be due to a depression of the sugar-output by the liver, to increased sugar-retention or destruction by the muscles, to leakage of the sugar into the secretions, or to destruction of the sugar in the blood itself. Regarding the first possibility, there is some evidence of decrease in the sugar-output by the liver in Experiment II, but since no such evidence is forthcoming in the three other experiments of this group, increased retention of sugar by the liver cannot be considered as an important factor in explaining the decrease. Nor does increased retention of dextrose by the muscles appear to have occurred; indeed, on the contrary, in most of the experiments the blood sugar of the iliac vein after the injections had been continued for some time was somewhat higher than in that of the vena cava or portal vein. We are unable to offer any explanation of this unexpected result (loss of water?) The third possibility, namely, an escape of dextrose into the secretions, is supported by the lower percentage in the portal-blood than in either of the other bloods. This secretion may have occurred by way of the urine or intestine. The decreased glycosuria produced by alkali administration that has been observed by Murlin and Underhill would seem to support the idea that escape had occurred by the intestinal pathway.

*2. Comparative sugar-retaining ability of liver and muscles during the injection of moderate amounts of dextrose into the portal vein.* In two experiments (fig. 2) the sugar-content of blood removed from the portal vein, vena cava and iliac vein was determined at frequent intervals during the continuous injection of moderate amounts of sugar into the portal vein. In one (No. VIII) just enough sugar (viz., 0.14 grams per kilogram an hour) was injected in acid solution to cause a slight hyperglycaemia; whereas in the other (No. VII), the sugar was given in larger amounts (0.7 gram per kilogram an hour), and at intervals 10 cc. of a 15 per cent solution of sodium carbonate were injected through the pancreatico-duodenal vein. The results of the former experiment would seem to indicate that a slight degree of acidosis does not disturb the relationship of the sugar content of the blood from the three veins. In the latter experiment, the considerably larger, though not excessive, sugar injections caused the portal blood-sugar to rise above that of the vena cava, the muscle blood-sugar being distinctly lower. In this experiment it will also be observed that the H-ion concentration of the blood became raised in spite of the injection of considerable quantities of alkali. In both experiments the rise in blood-sugar was very distinct immediately following the injection (horizontal arrows), a practically constant level being attained after about thirty-five minutes when 0.7 gram dextrose per kilogram an hour was injected, and in about twenty minutes with 0.14 gram.

*3. Comparative sugar-retaining ability of liver and muscles during the injection of large amounts of dextrose into the portal vein.* The rapidity with which the excess of dextrose disappears from the blood when large amounts are suddenly injected intravenously has in general been found by previous workers to depend much more on retention of the dextrose by the muscles and other tissues than by the liver (Bang, Kleiner, etc.). Although there can be no doubt that the sudden flooding of the organism with dextrose in such experiments is to be considered as a highly abnormal condition, and one which cannot conceivably occur even during the severest forms of experimental hyperglycaemia, yet the results of the experiments have been thought to be of some value as indicating the manner by which *any* excess of dextrose in the blood might be dealt with. By comparison of the percentage of sugar in blood taken from the portal vein, the vena cava, and the iliac vein, we have studied the sugar-retaining powers of the liver and muscles during the continuous injection (by means of Woodyatt's apparatus) of from 2.4 to 3.5 grams dextrose per kilo body weight

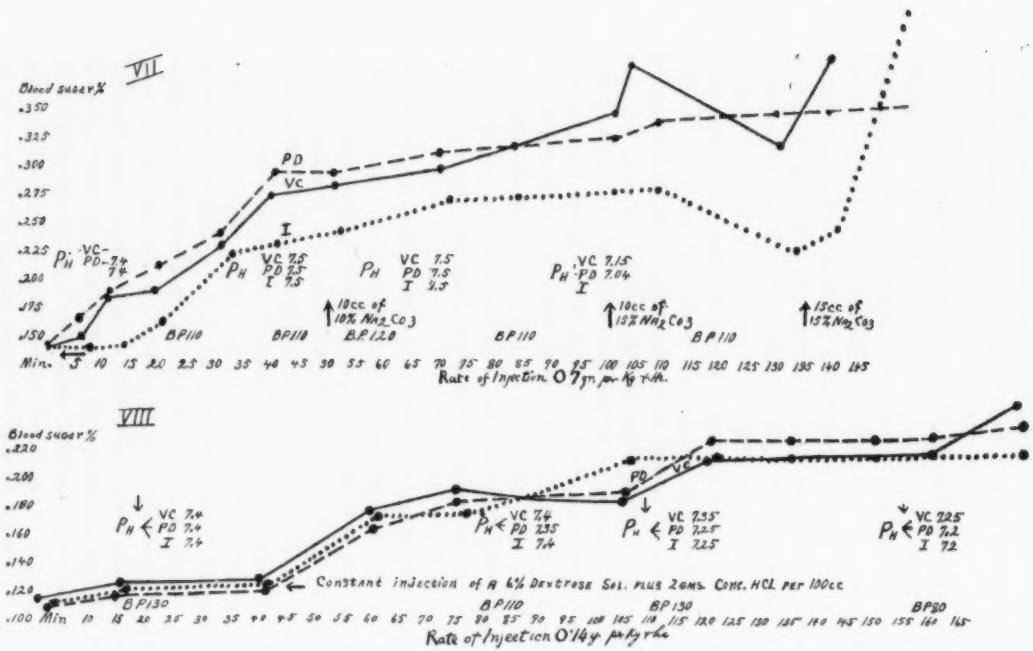


Fig. 2. Injections of moderate amounts of dextrose and of acid or alkali into the portal vein.

*Experiment VII.* Weight, 14.7 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Constant injection of a 6 per cent dextrose solution, 3 cc. per minute for 2 hours, 37 minutes, or 0.7 gram per kilo per hour. The vertical arrows indicate the time at which the alkaline solutions were injected.

*Experiment VIII.* Weight, 25.9 kg. Used Woodyatt's perfusion apparatus. Constant injection of an acid dextrose solution containing 2 grams of concentrated HCl per 100 cc. of a 6 per cent dextrose solution. Injected nearly 1 cc. per minute for 2 hours, 15 minutes, 0.14 gram per kilo per hour. Symbols same as in previous figures.

per hour. Acid or alkali was also injected at intervals through the pancreatico-duodenal vein. The results of the three experiments are depicted in the curves of figures 3, 4 and 5.

In Experiment XIII, figure 3, 3.5 grams dextrose per kilogram an hour was injected, causing a steady rise in blood-sugar in all three veins, most marked in the portal vein, next in the vena cava, and least in the iliac. As judged from the curves, the sugar-retaining power of liver and muscles is about equal. It will further be noted that, at intervals of about thirty minutes throughout the experiment, 10 cc. of 10 per cent sodium carbonate solution was injected through the pancreatico-duodenal vein, but that the H-ion concentration of the blood collected some minutes after several of these injections had been made did not show any increase in alkalinity; on the contrary, there was towards the end of the experiment a very distinct decrease in this regard, i.e., PH became lower. Since in our other experiments approximately similar quantities of alkali have caused an increase in blood alkalinity, we have in the results of this experiment some evidence that, when large quantities of dextrose are injected into the blood, acid breakdown products result—presumably lactic acid. In work which we hope to publish immediately we have, as a matter of fact, evidence of a striking increase in the lactic acid content of the blood when much smaller quantities of dextrose in alkaline solution are injected.

In Experiment XIV, figure 4, injections of 3 grams dextrose per kilogram an hour, accompanied by injection by way of the pancreatico-duodenal vein of 10 cc. quantities of hydrochloric acid, gave results which differed from those of Experiment XIII in that the muscles showed no evidence of retaining any dextrose; on the contrary, during a great part of the experiment there was more dextrose in the blood coming from them than in that of the vena cava. Although estimation of the H-ion concentration of the blood could not be carried out in this experiment, there is no doubt, for the reasons given above, that it must have been raised. The effect of this on muscular tissue may have merely been to raise its power of absorbing water, thus causing an increase in the sugar concentration of the outflowing blood. We were well aware of this possibility when the experiment was performed, and attempted to control it by enumeration of erythrocytes, but unfortunately in the rush of work entailed in such experiments, failed to carry through the process satisfactorily.

In Experiment XV, figure 5, 2.4 grams dextrose per kilogram an hour was injected without any acid until after a little over an hour,

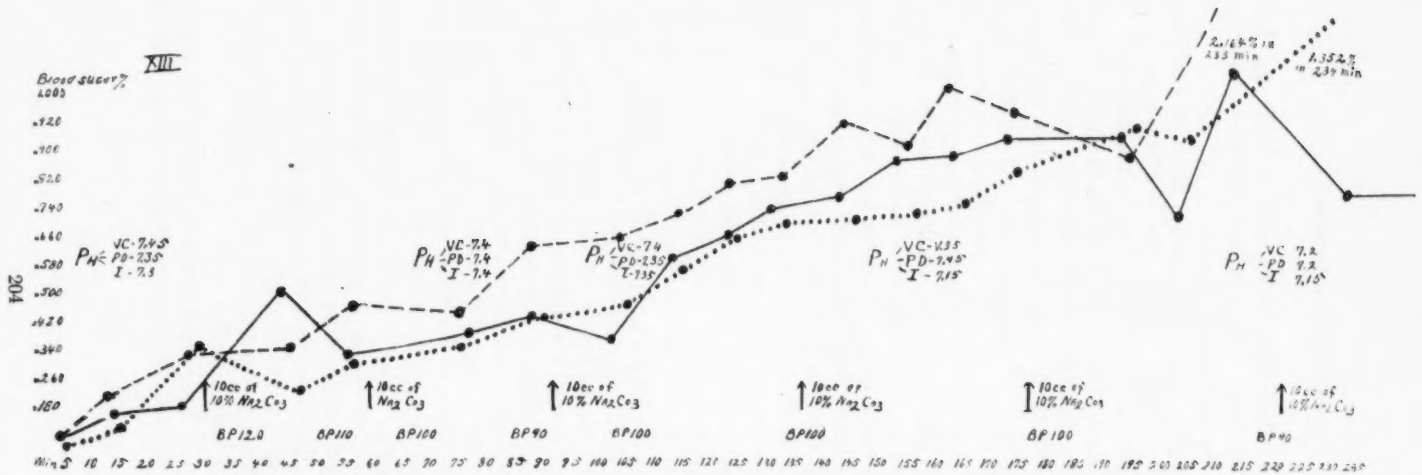


Fig. 3. *Experiment XIII.* Weight, 12.3 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Constant injection of an 18 per cent solution of dextrose, injecting 806½ cc. of the dextrose solution in 3 hours, 48 minutes, an average of 3.53 cc. per minute, or 3.5 grams per kilo per hour. At time indicated by vertical arrows $Na_2CO_3$ was injected into the pancreatico-duodenal vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I*, iliac, dotted line.

when hydrochloric acid in 10 cc. quantities was injected at about hourly intervals through the pancreatico-duodenal vein. It will be observed that the H-ion concentration of the blood did not rise except in that of the iliac vein. This is the only experiment in which large sugar-injections without *excess* of alkali did not cause a distinct decrease in PH. Regarding the behavior of the sugar, it will be observed that the liver and muscles apparently retained about equal amounts. Towards the end of this experiment a sudden fall occurred in the blood-sugar of

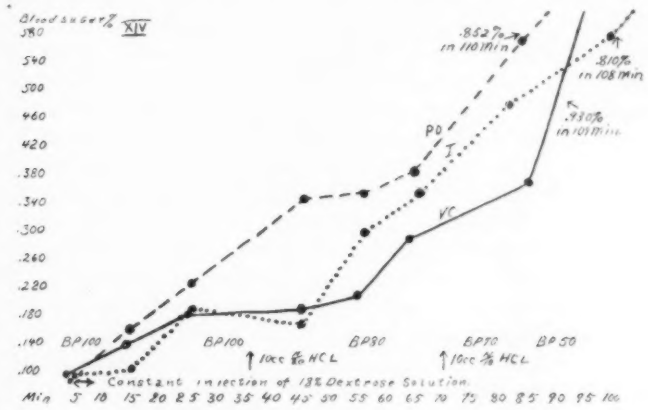


Fig. 4. *Experiment XIV.* Weight, 13.3 kg. Used Woodyatt's perfusion apparatus set at 2.5. Constant injection of an 18 per cent dextrose solution, injecting 3.8 cc. per minute for 1 hour, 30 minutes, or 3.08 grams per kilo per hour. At intervals specified above by the vertical arrows, 10 cc. of $\frac{N}{20}$ HCl and $\frac{N}{10}$ HCl was injected into the pancreatico-duodenal vein. *V.C.*, Vena cava, continuous line; *P.D.*, pancreatico-duodenal vein, broken line; *I*, iliac, dotted line.

all three vessels (not shown in the curve), although the sugar injections were being maintained. This fall occurred about the same time as a fairly rapid fall in arterial pressure and immediately following an injection of very strong acid. It would seem to indicate a sudden leakage of dextrose, perhaps because of the onset of marked oedema. In these three experiments it will be observed that a constant level of blood-sugar was never attained.

*4. The sugar-retaining ability of the liver during the injection into the portal vein of small quantities of dextrose in strongly alkaline as compared*

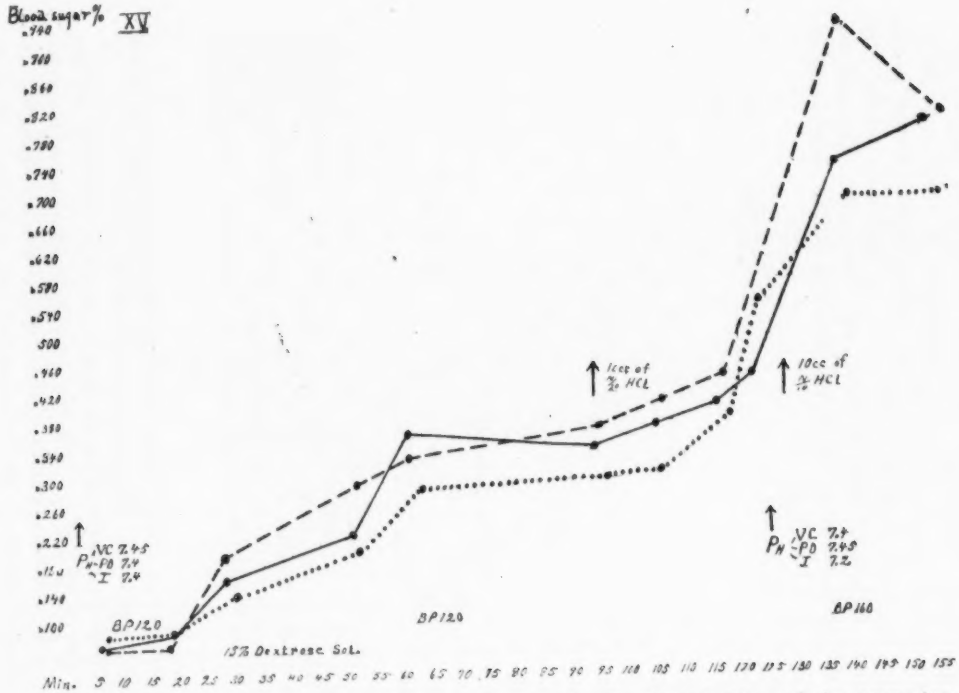


Fig. 5. *Experiment XV.* Weight, 17.2 kg. Kennel fed. Used Woodyatt's perfusion apparatus set at 2.5. Constant injection of an 18 per cent solution of dextrose. Injected 3.8 cc. per minute for 3 hours, 34 minutes, or 2.38 grams per kilo per hour. At intervals indicated by vertical arrows, acid was injected into the pancreatico-duodenal vein. *P.D.*, Pancreatico-duodenal vein, broken line; *V.C.*, vena cava, continuous line; *I.*, iliac, dotted line.

*with neutral or faintly acid reaction.* In the earlier experiments of this group (see fig. 6), the procedure consisted in collecting samples of blood from the vena cava and portal vein before and during the injection of a 4 per cent solution of dextrose into a mesenteric vein. The periods of injection were comparatively short, and the injections were made at a rate varying in the different experiments between 0.55 and 3.0 grams per kilogram an hour. The dextrose solution either contained from 10 to 20 per cent $Na_2CO_3$, or was neutral or faintly acid in reaction. Taking the results in general, it appeared as if the alkaline reaction caused a very striking sugar-retention as compared with that occurring with neutral or faintly acid dextrose solutions. Besides the table of results which we have already published elsewhere (14), the accompanying curves will show our reasons for coming to this preliminary conclusion. On closer examination of the curves, however, it will be observed that the above mentioned difference depends not so much on a falling away in the percentage of sugar in the cava blood as on the fact that the increase in the sugar-concentration of the portal blood produced by the injection of sugar solution is much more likely to occur suddenly when the latter is alkaline in reaction than when it is neutral or acid. When a similar rise in the portal blood does occur with neutral or acid sugar solutions, the liver retains just as much dextrose as it does in the presence of alkali. Another objection to the earlier experiments rests on the fact that the periods of injection were too brief, the one being also too quickly followed, sometimes without any no-injection interval, by the other.

As to the cause of the sudden increase in the portal blood sugar during alkali-dextrose injections, we are inclined to the belief that it was due to inequalities in injection rate, for although the same "constant-pressure" injection apparatus was employed as in previous work with 4 per cent dextrose solutions by one of us and R. G. Pearce, yet in the present experiments a lower pressure was employed, and this, along with the viscosity of the carbonate solutions, and perhaps on account of an action of the latter on the blood or blood vessels, produced a temporary block, which afterward suddenly gave way.

In any case we are certain that the marked increase observed in portal blood sugar in these experiments is due to some irregularity of injection rather than to any chemical action which the alkali might conceivably have in increasing the reducing power of the blood. Although at room temperature and for the brief period of time during which the alkali and dextrose solution stood prior to injection, we could

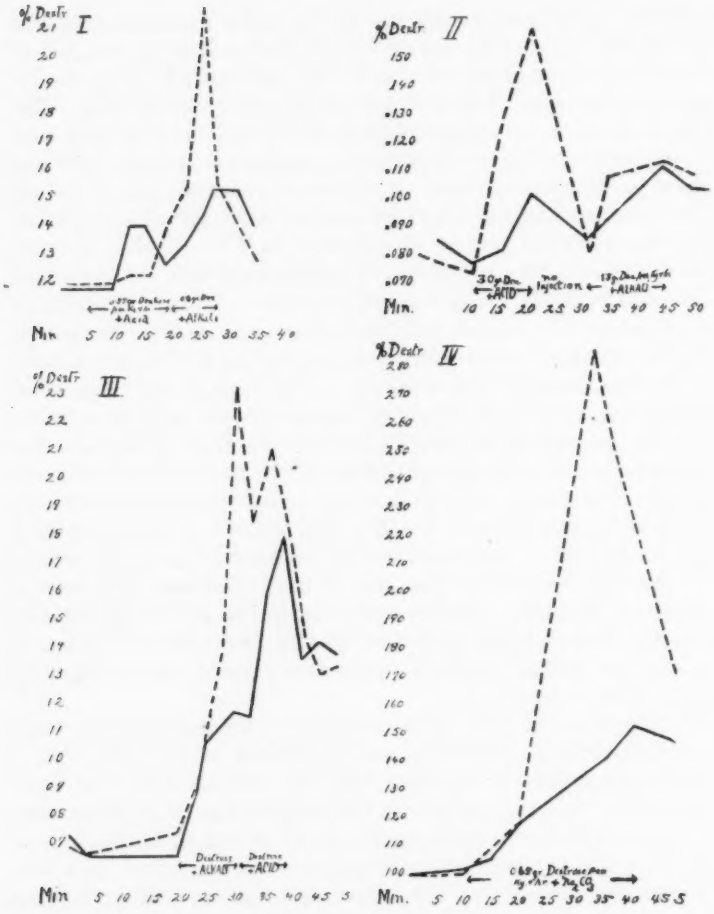


Fig. 6. The influence of alkali and acid on the blood-sugar in portal vein and vena cava during the injection of dextrose into the former. Broken line, blood of portal vein. Continuous line, blood of vena cava.

In I, 20 per cent $Na_2CO_3$ was injected at rate of 2.5 cc. per minute into portal.

In II, 5 per cent $Na_2CO_3$ was injected at rate of 7.5 cc. per minute and 0.5 per cent HCl at rate of 15.5 per minute into portal vein.

In III, 20 per cent $Na_2CO_3$ was injected, 8 cc. per minute. Weak (?) HCl at same rate.

In IV, 10 per cent $Na_2CO_3$ was injected, 2.6 per minute.

detect no change in its reducing power as measured by the Lewis-Benedict method, yet, since the changes described by Lobry de Brun, von Ekenstein and Nef must have set in, the possibility was considered as to whether the resulting mixture of hexoses, etc., might not, when mixed with blood, cause the production of strongly reducing substances. To test this possibility we added quantities of alkaline dextrose solutions similar to those used in the above injections to freshly drawn unclotted blood, and determined the reducing power after keeping the mixtures at body temperature for periods varying from a few seconds to over an hour. Since it is known that the products of the sugar-breakdown by alkali vary considerably according to whether or not oxygen is present, the above mentioned experiments were performed with both venous and arterial blood, the incubation in the former case being conducted in vacuo and in the latter, in the presence of air. It was found that, apart from slight differences in glycolysis, there was no difference in the sugar content of the various samples according to whether an alkaline or a neutral (or faintly acid) dextrose-solution was added to them.

We have repeated the injection experiments of this group, using the perfusion apparatus of Woodyatt, so that there could be no doubt as to constancy of injection, and with the modification that between the sugar-injection periods a sufficient interval was allowed to elapse during which the blood sugar could return to its normal level. The results are depicted in the curves of figure 7.

Taking as our criterion of the sugar-retaining power of the liver the difference between the portal and cava curves, it will be seen that with dextrose alone the percentage of blood-sugar in both veins is either the same (No. VI), or when the injection is somewhat greater, the portal vein sugar rises above that of the vena cava (No. VII). When acid sufficient to raise the H-ion concentration of the blood was given along with the dextrose, it was possible out of four experiments to demonstrate a greater percentage of sugar in the blood of the vena cava in only one case (No. IV). In the three other experiments of this type (Nos. II, III, V), the concentrations of sugar in the blood of these two veins were practically the same. Unfortunately in only one of these experiments (No. IV) was $P_H$ determined at sufficiently frequent intervals to show that enough acid was being injected to cause a perceptible increase in the H-ion concentration of the blood. Since however the animals were all of approximately the same size, and the same amounts of acid were injected in all four experiments, it may be inferred that the blood in all cases showed a distinct degree of acidosis.

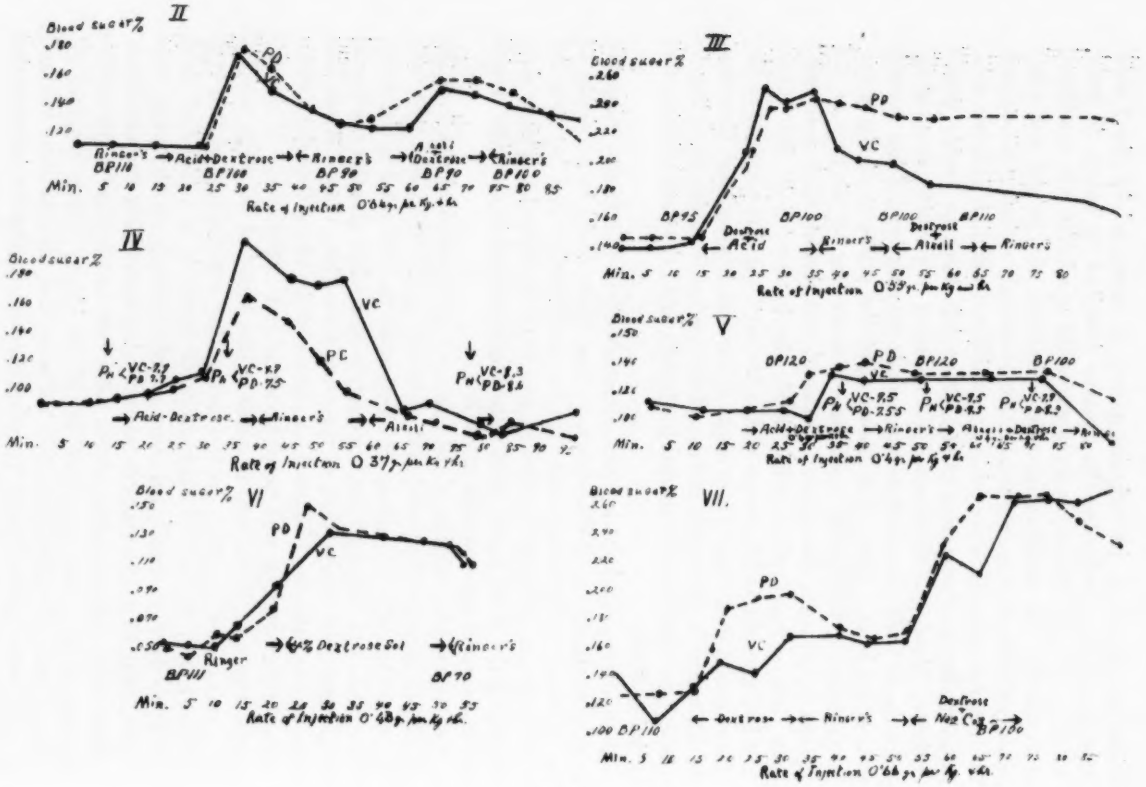
II
Blood sugar %
PD
VC
Ringer's BP 116
Acid → Dextrose BP 100
← Ringer's BP 90
Acid → Dextrose BP 90
← Ringer's BP 100
Min. 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Rate of injection 0·64 gm. per Kg. per hr.
III
Blood sugar %
PD
VC
BP 95
Dextrose Acid
BP 100
← Ringer's
BP 100
Dextrose
← Acid
BP 110
← Ringer's
Min. 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Rate of injection 0·55 gm. per Kg. per hr.
IV
Blood sugar %
VC
PD
Ph VC-8.8 PD 9.3
Ph VC-8.9 PD 9.5
Ph VC-8.3 PD-8.6
→ Acid-Dextrose → Ringer's → Acid
Min. 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Rate of injection 0·37 gm. per Kg. per hr.
V
Blood sugar %
BP 120
PD
VC
BP 120
BP 100
Ph VC-9.5 PD-9.55
Ph VC-9.5 PD-9.5
Ph VC-8.9 PD-8.9
→ Acid-Dextrose → Ringer's → Acid → Dextrose
Min. 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Rate of injection 0·49 gm. per Kg. per hr.
VI
Blood sugar %
PD
VC
Ringer
BP 111
→ ½% Dextrose Sol
← Ringer's
BP 90
Min. 5 10 15 20 25 30 35 40 45 50 55
Rate of injection 0·48 gm. per Kg. per hr.
VII.
Blood sugar %
PD
VC
BP 110
← Dextrose → Ringer's
Dextrose Na2 Co3
BP 100
Min. 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
Rate of injection 0·66 gm. per Kg. per hr.

Fig. 7.

Experiments IV and V are strictly comparable in practically every detail and yet in the only one of them is there evidence that the acid injection stimulated the glycogenolytic process of the liver sufficiently to make the concentration of sugar in the cava blood greater than that in the portal in spite of sugar injections into the latter. When acid is injected without sugar, the results are similarly irregular (i.e., sometimes only does the cava blood-sugar overstep that of the portal). The conclusion which we draw from these results is that it is impossible to demonstrate with certainty in anaesthetised animals that increased acidity of the blood supplying the liver excites this organ to more active glycogenolysis.

Turning now to the influence of alkali which was investigated in five of the experiments, it will be noted in the first place that the sudden increases found to occur in the portal blood-sugar in the earlier experiments (fig. 7) were not observed, thus indicating, as already remarked, that these must have been due to some irregularity in the injection. In three of the experiments (viz., II, III, and IV) when injected along with alkali, the dextrose caused a distinctly lower degree of hyperglycaemia to become developed than when injected in acid or neutral reaction. In a fourth experiment (viz., No. 5), this depressing

Fig. 7. The influence of acid and alkali, as compared with Ringer's solution, on the blood-sugar in portal vein and vena cava during the injection of dextrose into the former. Lettering the same as in figure 1.

*Experiment II.* Weight 14.1 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 6 per cent dextrose solution containing 3 grams of concentrated HCl per 100 cc., and (3) 6 per cent dextrose solution containing 15 grams of $Na_2CO_3$ per 100 cc.

*Experiment III.* Weight, 10.5 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution containing 2 grams concentrated HCl per 100 cc., and (3) 4 per cent dextrose solution containing 15 grams of $Na_2CO_3$ per 100 cc.

*Experiment IV.* Weight, 16.1 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution plus 2 grams concentrated HCl per 100 cc. and (3) 6 per cent dextrose solution plus 15 grams of $Na_2CO_3$ per 100 cc.

*Experiment V.* Weight, 14.7 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's, (2) 4 per cent dextrose solution plus 2 grams concentrated HCl per 100 cc., and (3) 4 per cent dextrose solution containing 10 grams $Na_2CO_3$ per 100 cc.

*Experiment VI.* Weight, 17.2 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of Ringer's and 4 per cent dextrose solution.

*Experiment VII.* Weight, 13.5 kg. Kennel fed. Used Woodyatt's perfusion apparatus. Injection of (1) Ringer's solution, (2) 6 per cent dextrose solution, and (3) 6 per cent dextrose solution containing 10 grams of $Na_2CO_3$ per 100 cc.

influence of alkali was somewhat later in showing itself, and in the fifth, (No. VII), in which however the amount of dextrose injected came very close to the 0.8 limit set by Woodyatt, etc., it was absent.

There can be no doubt from these results that alkali causes injected sugar to disappear more rapidly from the blood than otherwise would be the case, but there is only one experiment of this group (No. III) in which this disappearance might be attributed to a stimulation of the glycogen-retaining ability of the liver.

## CONCLUSIONS

1. When dextrose is injected by way of the portal vein into anaesthetised dogs in amounts which are below the tolerance limit (about 0.8 grams per kilogram an hour), there occurs a rapid increase in the percentage of blood-sugar. This rise continues until a certain level is attained, after which the blood-sugar remains practically constant. The time at which the constant level is attained varies with the rate of injection; thus, with from 0.14 to 0.4 grams per kilogram an hour it was attained in twenty to twenty-five minutes (figs. 2 and 7), whereas with 0.7 gram it required about thirty-five minutes (fig. 2). When the injections are considerably above the tolerance limit, the increase in blood-sugar goes on steadily for a very long period of time (two or three hours). Unfortunately we can offer no data regarding the behavior of the curve for dextrose injections that are just above the tolerance limit, i.e., between 0.8 gram and 2 grams per kilogram an hour.

2. When the behavior of the sugar concentration is compared in blood drawn from the portal vein, vena cava and iliac veins during moderate dextrose injections, it has usually been found that there is very little difference. The sugar-retaining powers of the liver and muscles are therefore not sufficiently developed to cause any perceptible change in sugar-percentage in the inflowing and outflowing blood of these tissues when physiological quantities of dextrose are being absorbed. Although such retention is no doubt going on, it is too slow in relation to the large volume of blood circulating through the tissues to make any impression on the sugar-percentage. When large injections of dextrose are given, a distinct difference in sugar-concentration usually becomes evident, the higher percentage being in the blood of the portal vein, the next in that of the vena cava, and the lowest in that of the iliac vein, and the difference between the concentrations of blood-sugar in portal vein and vena cava is usually about the same as that in vena cava and iliac vein. This suggests

that the sugar-retaining power of the liver is approximately equal to that of the muscles of the hind limb.

3. During the injection of large amounts of dextrose the H-ion concentration of the blood, as determined by the colorimetric method, very commonly becomes increased. When alkali ($Na_2CO_3$) is injected intravenously in sufficient amount to lower the H-ion concentration of the blood, in animals in whom the blood-sugar percentage is initially high (etherization and operative manipulation in sugar-fed dogs), there is a very distinct decrease in blood-sugar, but by comparison of the curves which represent this in the portal vein, vena cava and iliac vein, it cannot be shown whether the liver or the muscles are primarily responsible for the increased retention. The three curves decline at an equal rate.

4. The injection of sodium carbonate solution or of hydrochloric acid at intervals into the portal vein during the continuous injection into another branch of the same vein of a dextrose solution does not disturb the relationship in sugar-concentration normally existing in blood taken from portal vein, vena cava and iliac vein, nor do such injections cause any alteration in the level of the curves, whether these be rising or stationary. When dextrose is injected at a rate below the tolerance limit, along with sufficient alkali to cause a distinct increase in Ph of the blood of the portal vein and vena cava, the blood-sugar does not rise to such a degree as it does when the dextrose solution is neutral or acid in reaction.

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# THE RESPONSE OF THE VASOMOTOR MECHANISM TO DIFFERENT RATES OF STIMULI[1]

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Two effects are commonly observed upon stimulating the central end of a cut sensory nerve; (1) reflex pressor or vasoconstriction, (2) reflex depressor or vasodilation. Hitherto, considerable attention has been paid by investigators to the effect that different strengths of stimuli have upon the vasomotor center but few noted the effect that different rates of stimuli have upon this same mechanism.

Since it is possible to produce two effects, opposite in character upon stimulating the same sensory nerve, experimenters proceeded to find methods by which they could isolate the one or the other response. Latschenberger and Deahna (1) in 1816 were the first to obtain a fall in arterial pressure upon stimulating the central end of a cut sensory nerve other than the depressor from the aorta and heart. From their results, they attributed this fall in blood pressure to the use of weak stimuli and to certain changes in the vasomotor center itself. A fall in blood pressure was also recorded by Knoll (2) in 1885 upon stimulating sensory nerves. The results of his experiments led him to believe that a depressor response was usually the result of a weak stimulus whereas the pressor response followed, as a rule, a stronger stimulus. Upon stimulating muscles mechanically, by massage, Kleen (3) remarked a fall in general blood pressure.

Howell, Budgett and Leonard (4) were able to obtain reflex vasodilator and reflex vasoconstrictor responses from the same nerve trunk by cooling the nerve to 0°C. central to the point of stimulation. A fall in pressure was always the result of this procedure. This method, along with others, was also employed by Hunt (5). He obtained, upon stimulation, depressor responses from all sensory nerves tested,

[1] Reported at the 1915 meeting of the American Physiological Society. This Journal, 1916, xl, 136.

except the saphenous, in the cat; (a) when the nerves were cooled to 1°C.; (b) when the nerves were cut and recently regenerated; (c) when mechanical stimuli were applied to muscles; (d) when the electrical stimuli were weak; and (e) when the nerves were subjected to repeated stimulation over a long period. His experiments were recently repeated by Vincent and Cameron (6) who obtained similar results. They attribute a part of the fall in blood pressure to the respiratory movements. A simple inductorium was used in these experiments and the strength of the stimulus was increased or decreased by increasing or decreasing the number of cells in the primary circuit.

A fall in blood pressure was noted by Martin and Lacey (7) in all the experiments performed, except one. The success in obtaining depressor responses, they attributed to the use of weak or threshold currents.

Stimuli applied peripherally to severed nerves arouse the one or the other vasomotor response according to the rate of interruption of the stimulating current. Ostraumoff (8) observed that when the rate of stimulation was slow there resulted a rise in the temperature of the limb (vasodilation) but when rapid there was observed a fall in temperature (vasoconstriction). Similar results were observed by Kendall and Lucksinger (9). Bowditch and Warren (10) plethysmographed the limb and observed that a slow rate 1 to 4 stimuli per second was favorable to vasodilation and 30 to 64 stimuli per second favorable in producing vasoconstriction. Bradford (11) remarked similar results upon the vasomotor nerves to the kidney.

Since different rates of peripheral stimuli are favorable for isolating the different vasomotor responses, is it not possible that such changes could be effected reflexly? Few investigators applied different rates of stimuli centrally to sensory nerves when testing vasomotor reflexes. Hunt (5) is, as far as I know, the only investigator employing different rates who states the resultant responses. He employed rates varying from 1 per two seconds to 60 per second. In his research, the rate of stimulation did not alter the character of the vasomotor response. Martin and Lacey (7) tried rates of stimuli varying from 2 to 8 per second with one form of apparatus and from 4 to 60 per second with another. They, however, do not state which of these rates was commonly employed, nor do they state the effects observed upon changing the rate.

This research was undertaken to determine (a) whether or not the rate of stimulation has an effect upon the reflex vasomotor mechanism;

(b) whether or not the marked uniformity in the depressor effects observed by Martin and Lacey (7) may be due not only to the fact that they employed weak or threshold currents but also that they employed slower rates than did previous investigators.

## THE METHOD

Cats, narcotized with urethane (2 grams per kilo, by stomach), were used in these experiments. A few experiments were also performed upon cats under ether anaesthesia. The skin was incised on the median line of the neck, and the animal tracheotomized. The blood pressure was registered usually from the right carotid but in a few cases from the femoral artery by means of a mercury manometer. A signal magnet, which marked intervals of five seconds, was placed at the atmospheric pressure line of the manometer. It also indicated in most cases the time of stimulation.

For sensory stimulation the saphenous, peroneal, ulnar, radial, median and popliteal nerves were used. By making a small slit through the skin, at the position of the nerve, the nerve was isolated, cut, and the central end fastened in a Sherrington (12) shielded electrode. The electrode was then held in place by fastening around it, with paper clips, the two flaps of skin. The nerves were always used for stimulation in the order named above.

## THE STIMULATING CURRENT

*1. The strength.* The strength of the stimulating current was usually 0.1 ampere in the primary circuit, and the strength of the secondary current was determined in *Z* units according to the Martin (13) method. In a few cases 1.0 ampere in the primary circuit was used. Since the electrodes were not altered after once in place and comparative results were desired this method was thought to be sufficiently accurate. Tissue resistance and $\beta$ units were, therefore, ignored.

*2. The rate.* A glass knife blade key (13) moved back and forth by a motor, made and broke the primary circuit uniformly one to twenty times per second. A slower rate 1 per two seconds was obtained by interrupting the current by hand with the same key. No attempt was made to short circuit the make shock. This alternating effect was thought to overcome any polarization which might take place in the nerve trunk at the point of stimulation.

## RESULTS

For convenience, the results are collected under three general heads according to the strength of the stimulus used. In table 1 the strength of stimulus is weak, it is about the same as that employed by Martin and Lacey (7): 5.8 to 17 Z units. Moderate strengths were employed in table 2. These varied from 32 to 224 Z units. In table 3 one strength of current, 494 Z units, was used.

TABLE 1

*Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 16 cats under urethane anaesthesia. Strength of stimulus 5.8 to 17 Z units*

| NERVE | NUMBER OF READINGS | AVERAGE Z UNITS | RATE OF INTERRUPTION OF CURRENT PER SECOND | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 4 | 8 | | | 20 | | | | |
| | | | − | − | −+ | +− | − | −+ | +− | + | 0 |
| Saphenous | 15 | 8.4 | 15 | 11 | 3 | 1 | 3 | 5 | | 7 | |
| Peroneal | 18 | 12.2 | 18 | 17 | 1 | | 3 | 8 | 1 | 5 | 1 |
| Ulnar | 7 | 13.1 | 7 | 7 | | | 4 | 2 | | 1 | |
| Radial | 2 | 9.7 | 2 | | 2 | | | | | 2 | |
| Median | 3 | 13.5 | 3 | 3 | | | 1 | 2 | | | |
| | 45 | 11.2 | 45 | 38 | 6 | 1 | 11 | 17 | 1 | 15 | 1 |
| Per cent | | | 100 | 84 | 13 | 3 | 25 | 38 | 2 | 33 | 2 |

In this and following tables − fall in blood pressure; + rise in blood pressure; −+ fall followed by a rise; +− rise followed by a fall; 0 no change.

In table 1, 45 serial readings were made upon 16 animals. In this series of experiments at 4 interruptions per second 100 per cent of the experiments gave a pure fall in arterial pressure. This per cent was decreased to 25 upon changing the rate of stimulation from 4 to 20 interruptions per second. Usually the fall produced by 20 interruptions per second was less than that at 4 per second. With 20 interruptions per second 33 per cent of the experiments presented a pure rise in blood pressure and 40 per cent of the experiments showed a fall either preceded or followed by a rise in arterial pressure (see table 1).

The data, 51 serial readings, in table 2 were collected from 21 animals. There is in this table a greater range in strengths of stimuli, than in table 1, 32 to 224 Z units. However, the reactions are similar. With a rate of interruption of 4 per second 90 per cent of the readings gave a

pure fall, 6 per cent a fall followed by a rise and 4 per cent a pure rise in arterial pressure. With 20 interruptions per second 21 per cent of the readings presented a fall, 24 per cent a fall followed by a rise and 55 per cent of the readings gave a pure rise in blood pressure (see table 2).

Slower rates were employed in table 3. In most instances 1 per second was employed for the slowest rate in the series but occasionally 1 per two seconds was used. With this rate of stimulation 93 per cent of the readings presented a fall, 5 per cent a fall followed by a rise and 2 per cent a rise in arterial pressure, with the rapid rate of interruptions—20 per second—only 2 per cent gave a fall in blood

TABLE 2

*Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 21 cats under urethane anaesthesia. Strength of stimulus 32 to 224 Z units*

| NERVE | NUMBER OF READINGS | AVERAGE Z UNITS | RATE OF INTERRUPTION OF CURRENT PER SECOND | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 4 | | | 8 | | | | 20 | | |
| | | | − | −+ | + | − | −+ | + | 0 | − | −+ | + |
| Saphenous | 13 | 49 | 13 | | | 8 | 4 | 1 | | 2 | 3 | 8 |
| Peroneal | 25 | 67 | 21 | 3 | 1 | 12 | 9 | 4 | | 7 | 3 | 15 |
| Ulnar | 9 | 135 | 8 | | 1 | 7 | | 1 | 1 | 1 | 4 | 4 |
| Median | 4 | 79 | 4 | | | 3 | 1 | | | 1 | 2 | 1 |
| | 51 | 82 | 46 | 3 | 2 | 30 | 14 | 6 | 1 | 11 | 12 | 28 |
| Per cent | | | 90 | 6 | 4 | 60 | 27 | 11 | 2 | 21 | 24 | 55 |

pressure; 11 per cent of the readings presented a fall followed by a rise, 11 per cent a rise followed by a fall and 76 per cent a pure rise in blood pressure (see table 3).

Some curves illustrative of the results obtained upon varying the rate of the stimulus are here presented. Figure 1 is a curve in which weak or threshold stimuli were used. In this record the strength of stimulus was 5.8 Z units. At (*1*) the saphenous nerve was stimulated at the rate of 20 per second. There resulted during the time of the stimulus (approximately 30 seconds) a rise of arterial pressure from 96 to 99 mm. of mercury—a rise of 3.1 per cent. At (*2*) the rate of stimulation was 8 per second. At (*3*) the rate of interruption was 4 per second and there resulted a fall in arterial pressure from 98 to 94 mm. of mercury—a drop of 4.1 per cent.

TABLE 3

*Blood pressure changes induced by altering the rate of stimulation on sensory nerves in 24 cats under urethane anaesthesia. Strength of stimulus 494 Z units*

| NERVE | NUMBER OF READINGS | RATE OF INTERRUPTION OF CURRENT PER SECOND | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1* | | | 4 | | | | 8 | | | | 20 | | | | |
| | | − | −+ | + | − | −+ | +− | + | − | −+ | +− | + | − | −+ | +− | + | |
| Saphenous | 14 | 13 | 1 | | 5 | 7 | 1 | 1 | 1 | 6 | 1 | 6 | 1 | | 1 | 12 | |
| Peroneal | 22 | 21 | 1 | | 8 | 5 | 4 | 5 | 3 | 1 | 2 | 16 | | 3 | 3 | 16 | |
| Ulnar | 12 | 10 | 1 | 1 | 4 | 5 | | 3 | 3 | 1 | 1 | 7 | | 2 | 1 | 9 | |
| Median | 5 | 5 | | | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | | 1 | 1 | 3 | |
| Popliteal | 2 | 2 | | | 1 | 1 | | | | 1 | | 1 | | | | 2 | |
| Radial | 1 | 1 | | | | 1 | | | | | | 1 | | | | 1 | |
| | 56 | 52 | 3 | 1 | 19 | 20 | 6 | 11 | 8 | 10 | 5 | 33 | 1 | 6 | 6 | 43 | |
| Per cent | | 93 | 5 | 2 | 33 | 36 | 11 | 20 | 14 | 18 | 9 | 59 | 2 | 11 | 11 | 76 | |

* In some cases 1 per two seconds.

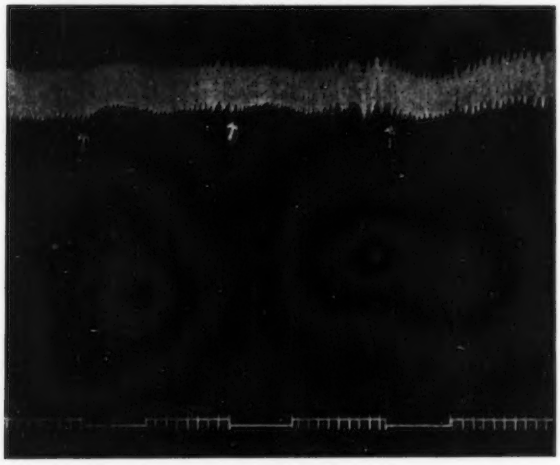

**Fig. 1.** Upper curve blood pressure. The lower line time in five seconds and the point of stimulation (also zero blood pressure). Saphenous nerve 5.8 Z units. This and all following records are explained in the text.

Figure 2 is a type of curve in which pure pressor and depressor results are observed. In this record the strength of stimulus applied to the saphenous nerve was 32 Z units. Similar results were obtained upon this animal with as weak a current as 17 Z units. From this curve the striking difference in the effects that slow and rapid rates have upon the vasomotor mechanism can be seen. At (*1*) the rate of stimulation was 4 per second and applied approximately 30 seconds. The arterial pressure decreased from 117 to 106 mm. of mercury—a fall of 9.4

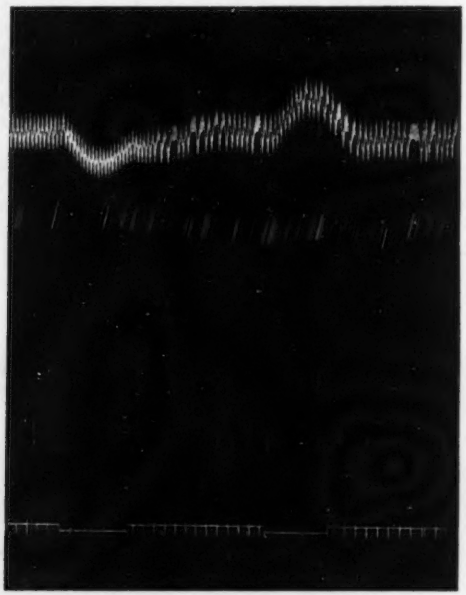

Fig. 2. Upper curve blood pressure. Lower line zero pressure, time in five seconds. Stimulation of saphenous nerve.

per cent. At (*2*) the rate of stimulation was 20 stimuli per second and the blood pressure rose from 115 to 127 mm. of mercury—a rise of 10.4 per cent. Similar results were obtained upon other animals not only with moderately weak currents but also with threshold currents and with stimuli up to 283 Z units.

A reversal of the blood pressure is not always obtained upon increasing the rate of interruption but a fall produced by a rapid rate is usually

less than that brought about by a slower rate of stimulation. Figure 3 is a curve obtained by stimulating the ulnar nerve with a strength of stimulus of 88 Z units. At (*1*) the rate of stimulation was 4 per second and there resulted a fall of blood pressure from 116 to 102 mm. of mercury—a drop of 12 per cent. Twenty interruptions per second were used in (*2*) and there resulted a fall of 2 mm. of mercury—a drop of about 2 per cent. Seven interruptions per second were used in (*3*) and 4 interruptions in (*4*). In the former case there resulted a fall of 4 per cent and in the latter a fall of 8.5 per cent.

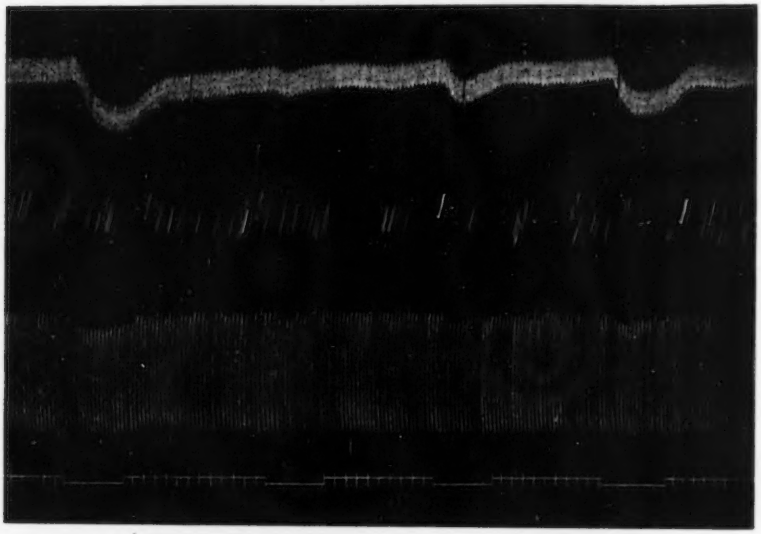

Fig. 3. Upper curve blood pressure. Middle curve respiration. Lower line zero blood pressure, time in five seconds. Stimulation of ulnar nerve.

Figure 4 is another type record obtained upon stimulating the peroneal nerve with a strength of stimulus of 32 Z units. At (*1*) the rate of stimulation was 7 per second and a fall in blood pressure is observed. In this curve as in other curves in which the rate of the stimulus was favorable to dilation, the pressure falls and rises slowly. When the rate is faster the curve falls abruptly and ascends rapidly and may reach the normal level before the stimulus has ceased. At (*2*) the rate of stimulation was 20 per second and there is seen a slight fall in blood

pressure followed by a rise. This type of curve was frequently observed when the stimulus was weak or moderately strong. As the currents became stronger the curves were usually the reverse, that is, a rise was usually followed by a fall in blood pressure.

Figure 5 is a record obtained by stimulating the ulnar nerve with a strength of stimulus of 494 Z units. At (*1*) the rate of stimulation was 1 per second and there resulted a fall in arterial pressure of 13 per cent. At (*4*) the rate of stimulation was 4 per second and at (*20*)

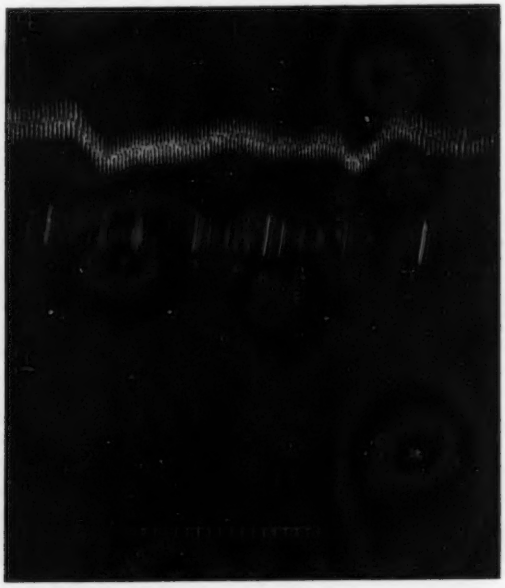

Fig. 4. Upper curve blood pressure. Lower line, atmospheric pressure, time five seconds, and point of stimulation.

20 per second, with a resultant rise in arterial pressure of 26 per cent in the latter case.

Hunt (5) summarizes his results on changes in the rate of stimulation upon the vasomotor center as follows, "Only a rise in pressure resulted, the character of which was determined by the number and strength of the stimuli; the rise was very gradual with slow weak stimulation, but took place much more suddenly with stronger and more rapid stimulation."

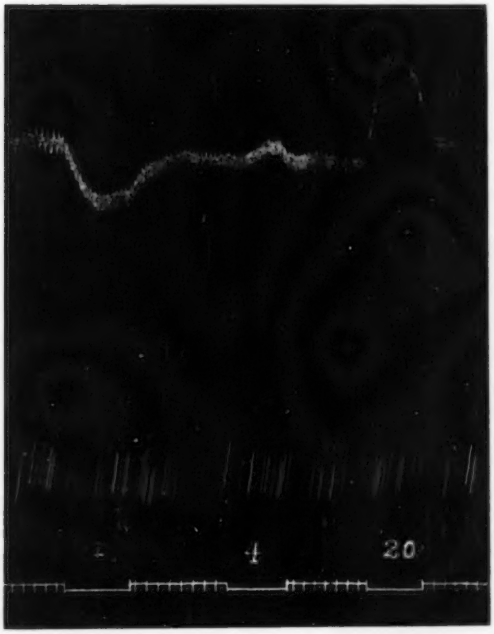


Fig. 5. Upper curve blood pressure. Lower line zero blood pressure, time five seconds, and point of stimulation.

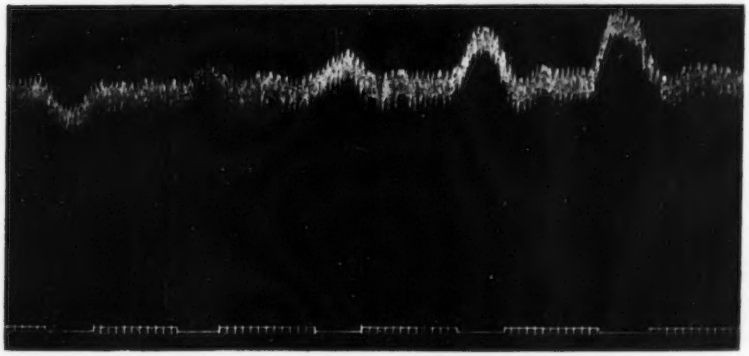

Fig. 6. Upper curve blood pressure. Lower line zero blood pressure, time in five seconds and point of stimulation.

The results obtained in figure 6 are slightly different from those observed by Hunt. The strength of stimulus applied to the peroneal nerve was 494 Z units. At (*1*) the rate of stimulation was 1 per second causing a fall of 11.3 per cent. At (*2*) the rate was 4 per second from which there resulted a rise of 7.1 per cent. At (*3*) the rate of stimulation was 8 per second producing a rise in arterial pressure of 11.2 per cent. A rise of 20 per cent was observed when the rate of stimulation was 16 per second (at *4*) and a rise of 28 per cent was obtained when the rate of stimulation was 20 per second (at *5*).

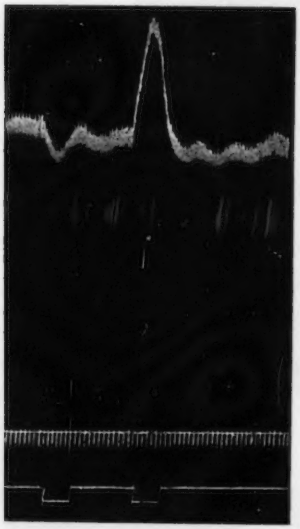

Fig. 7. Upper curve blood pressure. Middle line zero pressure and time in five-second intervals. The lower line the point of stimulation.

In a number of experiments a current of 650 Z units slowly interrupted (1 per second) gave a fall in pressure, whereas 4 per second produced a rise. In only two instances stronger currents were employed. In these cases 1 ampere was used in the primary circuit. One animal, on November 23, from which figure 7 is taken 2244 Z units produced a fall of 6 per cent when the rate of interruption was 1 per two seconds (at *1*) and a rise of 33 per cent when the rate of interruption was 4 per second (at *2*). A rise of 7 per cent in blood pressure was obtained on this same popliteal nerve, when the rate of interruption was 10 per second and the strength of stimulus was 244 Z units. Using the peroneal nerve of the same animal 8891 Z units interrupted at the rate of 1 per two seconds produced a fall of 5 per cent, and a rate of interruption of the stimulating current—20 times per second—produced a rise of 28 per cent. On November 26, similar results were obtained; 17 Z units at 20 interruptions per second produced a rise in blood pressure. The current was then increased in strength to 6643 Z units with a rate of 1 per two seconds a fall of 5 per cent resulted, when the current was 4 per second, a rise of 7 per cent was observed.

## DISCUSSION

From the above data it is shown conclusively that not only the strength but also the rate of stimulation is important in bringing about reflex vasodilator and reflex vasoconstrictor changes. With the same strength of stimulus pressor and depressor results are obtainable by varying the rate of stimulation from 1 to 20 stimuli per second. A rapid rate of stimulation which usually brings about vasoconstriction occasionally produces a fall in blood pressure, but this fall is almost always less than that produced by a slower rate of interruption. With a slow rate, 4 per second, depressor (reflex vasodilator) responses, and with a rapid rate, 20 per second, pressor responses have been observed with 5.8 Z units. Upon increasing this current to 383 Z units or seventy times 5.8 Z units these same phenomena have been observed. Upon increasing the current to 494 Z units usually the rate of 4 per second produced a rise in pressure but a slower rate 1 per second or 1 per two seconds produced a fall. Not only was it possible to bring about a fall in blood pressure with 1 per two second stimulation with 494 Z units, but the strength of stimulus could be increased to 6643 Z units and still a fall was observed. This current was 390 times stronger than the current necessary to produce a rise in blood pressure when interrupted 20 times per second. That summation does take place with rapid rates of stimulation is undisputable, but it does not seem probable in this case where the strength is more than 400 times threshold that the phenomenon of summation can explain the different effects obtained with these rates of 1 per two second and 20 per second interruptions.

Most observers believe the pressor response to be the "normal" and predominating response upon cutaneous sensory nerve stimulation and the depressor response unusual except upon alteration in the nerve tissue itself (14). Martin and Lacey claim that the depressor response is the natural response because the threshold is lower. My experiments throw no further light upon the subject than that the threshold may be the same for both pressor and depressor responses, depending upon the rate of stimulation. If, however, the rate of stimulation is slow and uniform, then the threshold for the depressor response is the lower as observed by Martin and Lacey (7). Their claim (7) that depressor responses are not unusual, upon sensory nerve stimulation, is supported by these results.

SUMMARY

1. The rate of stimulation modifies the responses obtained reflexly from the vasomotor mechanism, when the saphenous, peroneal, ulnar, radial, median and popliteal nerves are stimulated centrally.

2. These results confirm Martin and Lacey's conclusions that no difference in effect on blood pressure was observed when different sensory nerves were stimulated. Radial, ulnar, median, peroneal, saphenous and popliteal nerves gave depressor responses upon stimulating with a weak, slowly interrupted current (4 per second). Pressor responses were obtained upon these same nerves with weak currents but the rate of interruption was increased from 4 to 20 per second.

3. Slow rates (1 per second or 1 per two seconds) of stimuli act favorably in bringing about depressor responses even when the strength of stimulus is 494 to 8891 Z units or 90 to 400 times the threshold stimulus.

4. In 45 serial readings made upon 16 cats under urethane anaesthesia, the average strength of current was found to be 10.2 Z units. One hundred per cent of the readings showed depressor effects with a rate of 4 stimuli per second and 73 per cent showed a pressor effect with a rate of 20 stimuli per second.

With an average current of 82 Z units for 51 readings upon 21 cats, 96 per cent of the readings showed a fall in blood pressure with a rate of 4 stimuli per second and 79 per cent showed a rise in arterial pressure with a rate of 20 stimuli per second.

With a strong current 494 Z units for 56 readings upon 24 cats 98 per cent showed a fall in arterial pressure at 1 per second or 1 per two second stimulation. Ninety-eight per cent of the readings showed a rise in blood pressure when the rate of stimulation was 20 stimuli per second.

5. Whether pressor or depressor responses are the "normal" results upon sensory stimulation depends upon the rate of stimulation and the strength of the stimulus. The threshold for both may be the same if the rate is slow (4 per second) for depressor and rapid (20 per second) for pressor responses.

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# THE EFFECT OF TEMPERATURE CHANGES ON RHYTHM IN THE HUMAN ELECTROMYOGRAM

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## INTRODUCTION

The frequency of nerve impulses discharged from the central nervous system in voluntary and reflex contractions of the skeletal muscles presents a problem concerning which great difference of opinion is found among investigators. Piper[1] who has made the most extensive study of action currents in human muscles, has shown with a string galvanometer that when electrodes are applied to the skin over a skeletal muscle (e.g., a forearm flexor) in such a way that interference currents are minimized, a fairly regular series of action currents with a rhythm of about 50 per second accompanies voluntary contraction. He states that the electrical effect of voluntary contraction can be reproduced by stimulating the motor nerve with 50 induction shocks per second, and that the records made under the two conditions are quite similar.[2] He infers from this that the central nervous system sends to the muscle 50 impulses per second.

Buchanan[3] has repeated the observations of Piper, using a capillary electrometer instead of a string galvanometer. She dissents, however, from Piper's conclusion that the muscle reveals the rhythm of innervation. Her reasons are in the main two. In the first place, her attempt to duplicate the electromyogram of voluntary contractions by stimulating the median nerve of a human subject with 50 induction shocks per second, yields with her capillary electrometer a record differing from that of the voluntary contraction.[4]

[1] Piper: Elektrophysiologie menschlicher Muskeln, Berlin, 1912; Pflüger's Arch., 1909, cxxix, 145, etc.

[2] Piper: Arch. f. Physiol., 1910, 208.

[3] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 225.

[4] Loc. cit., 229 and 242, figs. 6 and 10.

Her second reason is based on an important series of experiments[5] with decerebrate frogs drugged with strychnine. She found that when the frog's gastrocnemius muscle was connected with the electrometer the records of the reflex spasm showed usually two distinct rhythms. There were large undulations with frequencies of from 3 to 14 per second, and superimposed upon the initial or ascending phase of each of these were smaller undulations with frequencies between 40 and 100 per second. The larger undulations she designates waves; the smaller, wavelets. By placing the gastrocnemius muscle in a moist chamber separate from the rest of the animal, except for an aperture to admit the nerve, she was able to raise and lower the temperature of the muscle without altering that of the nerve centers. The result of this procedure was to raise and lower correspondingly the frequency of the "wavelets" without changing that of the waves. The wavelet frequency at a muscle temperature of 7°C. varied from 40 to 54 per second, at 16°C. it lay between 100 and 110 per second. From this she argued that the wavelets do not correspond with any central nervous rhythm but indicate a rhythm intrinsic in the muscle. To show that the wavelet rhythm is completely independent of the nerve centers she altered the temperature of the spinal cord while keeping the temperature of the muscle constant, with the result that the frequency of the waves changed but not that of the wavelets. She believes that the undulations seen in the electromyogram of human muscles in voluntary contraction partake of the nature of the wavelets in her frog records rather than of that of the waves. Certainly their frequency would so suggest. She contends that as the "wavelet" rhythm is "of peripheral origin," we have no evidence that the central stimulus is discontinuous in its nature. She attempted in one case to extend her observations on change of temperature to human muscles by comparing the electromyogram of the same individual with the arm in an atmosphere at 13°C. in one case, and in an incubator at 35°C. in the other. She found no change of rhythm sufficiently marked to exceed the limits of variation resulting from the usual irregularity in the oscillations. It may be remarked here that the temperature-regulating powers of the circulation are probably sufficient to maintain a close approximation to normal temperature in the muscle fibers under such diversity of atmospheric temperature as was here employed. At least the change of temperature was probably insignificant compared to that imposed on the frog's muscles.

[5] Buchanan: Loc. cit., 213–221; cf. also Journ. Physiol., 1901, xxvii, 95.

Garten[6] in dealing with this problem recorded human electromyograms under stimulation of the motor nerve with a constant current, with an alternating current of 2200 cycles per second, and in voluntary contraction. He found approximately the same action current rhythm under all three conditions, which suggested that the rhythm. was intrinsically muscular and independent of innervation rhythm. But a comparison of the records obtained from the nerve and muscle of the rabbit, the nerve being in each case stimulated with a constant current, showed so close a correspondence of rhythm in the two tissues that he concludes[7] that the muscle probably affords a true record of the discharge of nerve impulses from the ganglion cells.

Piper[8] argues against Buchanan's view, contending that the characteristic rhythm of 50 per second in voluntary contraction must be determined by an equal frequency of nerve impulses because it is possible to raise the frequency of induction shocks applied to a motor nerve as high as 300 per second and still obtain a corresponding action current rhythm in the muscle. He argues that if the muscle is capable of responding separately to 300 nerve impulses a second it would not limit itself to 50 responses per second when subject to stimulation which is continuous or of a frequency too high for the muscle to follow. The argument appears at first sight convincing, but we believe we can show presently that it is not necessarily valid. Piper[9] has also reported an experiment on the progressive increase in frequency of rhythm accompanying progressive rise in temperature, but the experiment is not crucial since he raised the temperature of the entire animal (turtle), thus making the change common to both nerve center and muscle.

Dittler[10] has made an important contribution to the subject by recording with the string galvanometer the action current of the diaphragm of the rabbit in spontaneous respiration, and subsequently[11] the action current of the severed phrenic nerve under experimental conditions as nearly similar as possible. In these experiments the rhythm of the muscle corresponded with that of the nerve so closely that Dittler concluded that in general a muscle reproduces the rhythm of its central innervation. His evidence surely points strongly in that

[6] Garten: Zeitschr. f. Biol., 1909, lii, 555.
[7] Loc. cit., 562.
[8] Piper: Arch. f. Physiol., 1910, 209.
[9] Loc. cit., p. 216.
[10] Dittler: Pflüger's Arch., 1909, cxxx, 400.
[11] Dittler: Pflüger's Arch., 1910, cxxxi, 581.

direction, but it does not necessarily follow that the same correspondence obtains in the voluntary contraction of skeletal muscles.

Beritoff[12] has made some experiments with a string galvanometer on the maximum frequencies of response obtainable in nerve and muscle and on rhythm of reflex muscular responses in the frog. In discussing his results he gives an excellent review[13] of the controversy under consideration, and urges that the observed rhythm of muscle under central innervation should not be regarded as the specific rhythm of muscle exclusively, or as the rhythm of central innervation. Whether the recorded muscle rhythm in any given case is that of central innervation or is intrinsically muscular, depends, in his view, on whether or not the impulses are sent out from the center at a frequency higher or lower than the "specific rhythm" of the muscle under the conditions of the experiment. In this it seems to us he makes an important step toward a true comprehension of the problem; but we believe that in the light of certain properties of nerve and muscle recently established by Lucas and Adrian[14] the interpretation of the facts already on record together with the results of our experiments, will make the matters less confusing. To this we shall return in the discussion of results.

At all events, Buchanan's observations on the change of wavelet rhythm with change of temperature confined to the muscle is of fundamental importance. But the gap between a strychnine spasm in the decerebrate frog and the voluntary contraction of muscle in man is such as to render it highly desirable that observations similar to hers should if possible be made on human beings under conditions more like those obtaining in Piper's experiments. With this idea in mind we have performed a few experiments, using ourselves as subjects.

## METHOD

Our object was to record the action currents of human muscles in voluntary contraction under as widely varying temperatures as possible and with as little variation as possible in the temperature of the central nervous system. We thought it probable that some of the small muscles of the hand lying close to the skin could be cooled appreciably

[12] Beritoff: Zeitschr. f. Biol., 1913, lxii, 125.

[13] Loc. cit., 190–196.

[14] Lucas: Journ. Physiol., 1911, xliii, 46; Adrian and Lucas: Journ. Physiol., 1912, xliv, 114; Adrian: Journ. Physiol., 1913, xlvi, 384.

by immersion in ice water and would stay cool long enough to admit of taking a record after withdrawal. But it seemed desirable first to make some test of the question whether the lowering of temperature resulting from such immersion could penetrate appreciably through the subcutaneous tissues, for it is conceivable that the circulation might be such as to keep the muscles very near normal temperature even though the skin was thoroughly chilled.

For this test a basin was filled with water maintained at approximately 7°C. and set on a stool beside the subject. A thermometer was secured with adhesive plaster so that the bulb remained in contact with the palm of the hand. The hand was then held, palm up, over the water, but not touching it, till the thermometer had come to rest and the fluctuations due to slight involuntary motions of the hand had been noted. When the temperature was steady the hand was cautiously lowered, care being taken not to permit any shift in the contact of the bulb, till the back of the hand and most of the wrist were immersed in the cold water. But no water was allowed to touch the palm. The recorded temperature at the beginning of contact with cold water was 31°C.; in four minutes it had fallen to 30°C.; in fifteen minutes it had fallen to 28.1°C. Later similar partial immersion in hot water varying between 43°C. and 47°C. raised the temperature of the thermometer from 28.1°C. to 30.9°C. in twelve minutes. From this it is evident that an easily appreciable fall of temperature can be transmitted from the surface not merely to the interior of the hand, but through it and through the skin again to the opposite surface. There is no doubt that with complete immersion of most of the forearm as well as the hand a fall of temperature can be produced in the small muscles of the hand still greater than was shown in the thermometer by this experiment. It is impossible to form any exact estimate of the degree of cooling of a muscle by this procedure, but it seems reasonable to expect from an immersion of most of the forearm in water at 6° to 7°C. for fifteen minutes a fall of more than 5°C., and perhaps a fall of more than 10°C. This should suffice to reveal a change of rhythm if conditions in human muscles are such as might be inferred from Buchanan's experiments on the frog.

The string galvanometer used in these experiments was of the Cambridge type. A full description of the electrical connections and photographic recording apparatus has already been published.[15] The

[15] Forbes and Gregg: This Journal, xxxvii, 1915, 121–130.

arc lamp was used for illumination, and with it the simplified optical system. The 5000 ohm platinum string, hitherto designated "String C," was used throughout.

The electrodes used for leading off the action current through the skin were essentially the same as those described by Piper.[16] They consisted of small glass funnels with their small ends cut off to admit rubber stoppers, and with cat bladders fastened over the large ends so that they could be filled with zinc sulphate solution. Zinc rods thrust through the rubber stoppers were thus introduced into the solution to make connection with the galvanometer. The outer surface of the bladder was soaked in salt solution when applied to the skin. When used they were secured with the bladders in contact with the appropriate skin areas by leather wristbands in which had been cut round holes a little smaller than the large end of the funnels, the wristbands being strapped around the arm as tightly as was comfortable. A saturated solution of zinc sulphate was then poured into the funnel till the rubber stopper with the zinc rod could be inserted leaving no bubble of air inside. In this way were eliminated such electrical disturbances as might result from changes in the portions of the zinc rod in contact with the fluid, which might be caused by mechanical agitation.

The muscle selected as best adapted for this investigation was the first dorsal interosseous which presents a well defined mass near the surface of the hand between the thumb and forefinger. In leading off from this muscle a small funnel electrode only about 25 mm. in diameter was applied over the belly of the muscle, while a larger electrode was placed on the back of the hand near the little finger. Both were secured in place by a single wristband with a hole for each electrode, strapped around the hand and passing through the angle between the thumb and forefinger. Contraction was produced by placing the proximal phalanx of the forefinger against the edge of a heavy table and making a strong effort to adduct the finger, which motion was opposed by the table. Another method used was to press the tip of the thumb against the side of the forefinger and exert in this way a mutual pressure.

The results with this muscle were so satisfactory that we were encouraged to repeat the experiment, using the forearm flexors which have served for the majority of electromyograms made by Piper and others, and which yield larger and somewhat more regular galvanometric

[16] Piper: Pflüger's Arch., 1909, cxxix, 149.

excursions than the small muscle in the hand. In using this muscle group the arm was immersed in ice water to a point above the elbow. The electrodes were applied, one a large one of about 53 mm. diameter) over the flexor muscles about midway between the elbow and the wrist, the other over the tendons of these muscles at the wrist. Each was secured by a separate wristband.

The experimental procedure began with washing in warm water the skin over the muscle to be studied. The object was to minimize any error that might result from comparing records taken through the skin which had not been previously moistened, with records taken through well soaked skin. Such an error could hardly influence the main question, i.e., the frequency of action currents, since the only significant alteration would probably be that of skin resistance, and such a change would affect the magnitude but not the frequency of galvanometric excursions. But it seemed desirable, none the less, to make conditions as uniform as possible, for any marked change of amplitude would be of interest.

The electrodes were then applied and a series of records was obtained with both moderate and strong contractions, the muscle being at approximately normal temperature. Then the hand was immersed in a large jar of cold water for from fifteen to twenty minutes, the temperature of the water being kept between 4°C. and 9°C. by the occasional addition of cracked ice. When only the first dorsal interosseous muscle was to be studied the arm was only immersed to a point midway between the wrist and elbow; when the forearm flexors were studied a tall jar was used which permitted immersion to a point above the elbow. Immediately after this immersion the skin was rapidly wiped dry, the electrodes were reapplied, and another series of contractions was recorded as soon as possible. In every case the records were made about four or five minutes after the hand was withdrawn from the cold water. The skin over the entire area that had been immersed felt well chilled to the touch for a considerably longer period than this, and we believe that the muscle was at the time of the records nearly as far below its normal temperature as it was at the moment of withdrawal. There also persisted a marked loss of efficiency in the cooled muscles, evidenced by the difficulty experienced in attempting to write with the chilled hand.

The hand was then immersed in water as hot as could readily be borne, i.e., between 44° and 47°C., for a period of six or eight minutes, sometimes longer. With the aid of the circulation the warming proc-

ess is more rapid than the cooling process, and it seemed to us that this period sufficed to raise the temperature of the hand about as high as it was possible to raise it. Again the hand was withdrawn, the skin wiped, the electrodes reapplied and another series of records taken at a time varying from three to six minutes after withdrawal.

In two of the experiments the question of the change of body temperature was controlled by noting at frequent intervals the buccal temperature. In each case the temperature remained between 37°C. and 37.2°C. during the entire experiment. From this we may safely conclude that there was not enough change of temperature in the ganglion cells to account for any change of rhythm which might appear in the records

We encountered in one experiment a technical difficulty which it proved necessary to guard against. In one record certain oscillations occurred with more persistent regularity than is commonly seen in electromyograms; their frequency was only about 28 per second and the oscillations due to the action currents, being of higher frequency, were superimposed upon them. These oscillations were traced to the vibration of the building due to a circular saw in the machine shop two floors below. That this was possible was surprising as the galvanometer is mounted on a stand built solidly against the outside stone wall of the building. But by having the saw alternately running and still during a series of observations we were able to demonstrate the dependence on it of these oscillations.

## RESULTS

The records showed with perfect uniformity a decrease in frequency together with an increase in amplitude of the excursions whenever the arm was chilled, and in most cases a somewhat less pronounced increase in frequency with a decrease in amplitude when the arm was heated. To make an accurate quantitative estimate of these changes in frequency is difficult, for, as may readily be seen by examining any of the electromyograms here reproduced, or any of those published by Piper and Garten,[17] it is possible to find in the records of any single second's duration all intermediate grades of oscillation from the largest down to barely perceptible deviations in the course of the curve. In consequence, it is sometimes hard to decide what to count. Piper

[17] Piper: Elektrophysiologie menschlicher Muskeln, Berlin, 1912, 82–85, 97. Garten: Loc. cit., figs. 38 and 39, plate xvii.

has contended that a proper estimate of the true frequency of action currents should be made by counting only the "primary waves"[18] and thus avoiding confusion due to "interference."[19] In our experiments with the forearm flexors we have been careful to place the

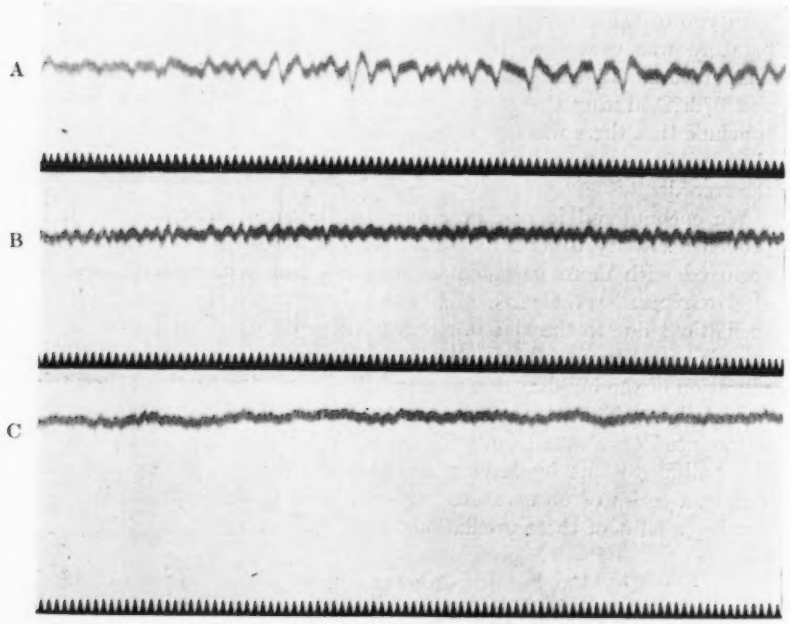


Fig. 1. Electromyograms of first dorsal interosseous muscle; adduction of forefinger against table. Experiment 3; Subject R. *A*, after fifteen minutes' immersion at 7°C. *B*, after four minutes' preliminary immersion at 40°C. *C*, after final immersion, fifteen minutes at 47°C. In all figures the upper line recording movements of the string, shows by upward excursions negativity in the electrode placed over the belly of the muscle. The lower line records time; each complete vibration = 0.01 second.

electrodes in positions corresponding closely to the points Piper designates "3" and "5,"[20] thereby eliminating as far as possible the secondary oscillations which he ascribes to "interference." In experi-

[18] Piper: Arch. f. Physiol., 1910, 211.
[19] Piper: Pflüger's Arch., 1909, cxxix, 157, fig. 3; Elektrophysiologie, etc., 29–32.
[20] Piper: Op. cit., 22; also Pflüger's Arch., loc. cit., 150, fig. 1.

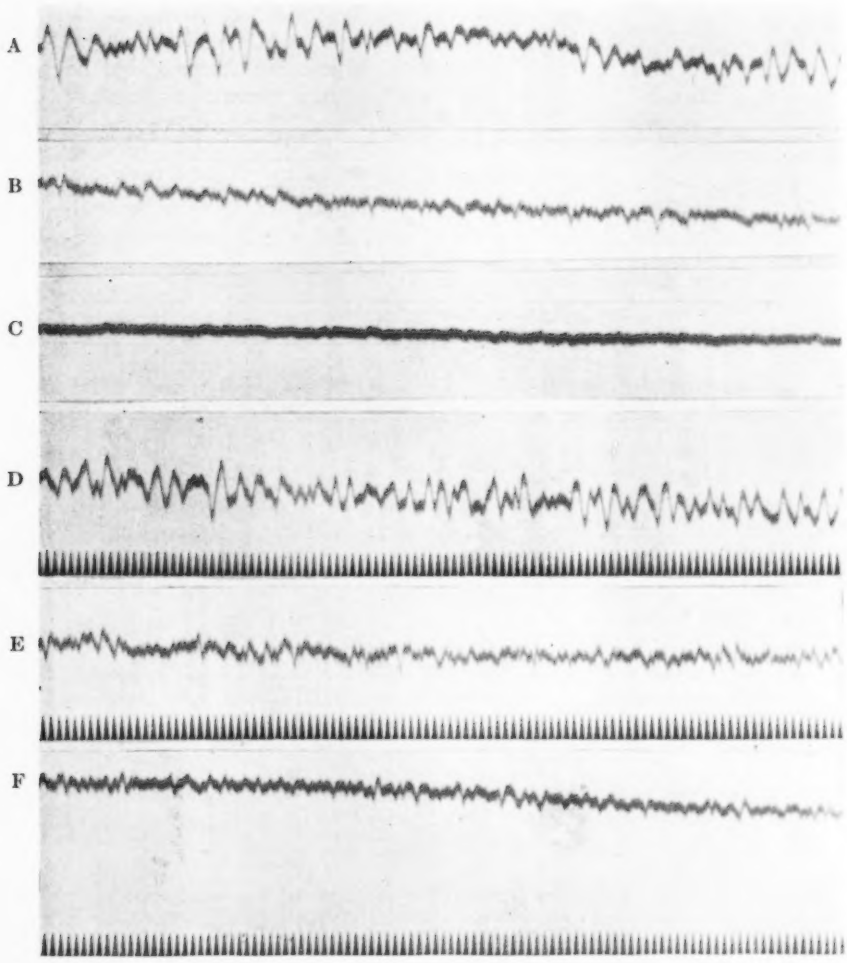


Fig. 2. Experiment 5; Subject F. First dorsal interosseous. *A*, *B* and *C* are records of adduction against table. *D*, *E* and *F* were made by pressure between thumb and forefinger (see text). *B* and *E* were taken first at normal temperature, *A* and *D* in quick succession after eighteen minutes' immersion (with a short interruption) at 4° to 5°C. *C* and *F*, after subsequent six minutes' immersion at 47°C. In this figure and in figures 3 and 4 some of the time lines have been omitted to save space, but in each experiment the speed of the film was constant in all records.

ments with the first dorsal interosseous the small size of the muscle has left little choice in the placing of the electrode intended to be at the focus of activity; and the position of the other electrode, on the back of the hand, should, as far as we can judge, serve as well as any other to render the oscillations simple in form. Yet small secondary notches appear and it is difficult to make a reliable distinction between these and the "primary waves" on whose importance Piper has insisted.

In spite of this difficulty we have found it possible to make estimates of relative frequency of oscillations, and whenever the tempera-

TABLE 1

| | COLD | | NORMAL | | HOT | |
|---|---|---|---|---|---|---|
| | Primary | Total | Primary | Total | Primary | Total |
| Experiment 2; Subject F. | 44 | 80 | 55 | 112 | | |
| | 45 | 89 | | | | |
| Experiment 3;† Subject R. | 36 | 69 | 63 | 104 | (65) | 137 |
| | 35 | 73 | 66 | 118 | | |
| Experiment 5; Subject F. | 40 | 74 | 50 | 107 | (64) | (114) |
| | 35 | 73 | | | | |
| | *38 | 81 | 48 | 111 | 60 | 132 |
| | 41 | 79 | | | 63 | 133 |

† In Experiment 3 the forearm was immersed in water at 40°C. for four minutes before the first records were taken. Thus the temperature of these, listed under "Normal," was slightly higher than normal. A correspondingly high count is to be noted.

* The figures in the last two lines are from records made by mutual pressure between thumb and forefinger. All the rest are from records of pressure against the table.

ture has been unchanged through two successive observations the uniformity of the estimates has justified them. The method was as follows: A length on the record corresponding to a second of time was marked off, a portion being chosen which showed a well sustained period of activity. A count was then made of major excursions, the criterion being that which seemed most nearly to make the count represent the prevailing rhythm. Then a second count was made for the same second, but this time every notch which could be detected with certainty was counted. The two figures were placed in separate

columns and the records at different temperatures were compared separately on each basis. Table 1 shows a comparison of these figures taken from records of the interosseous muscle; in the columns marked "Primary" are the counts of major excursions in a second; in the column marked "Total" are the counts of all discernible oscillations. In records made after heating the arm the smallness of the excursions renders difficult a satisfactory count, and some of these figures are therefore in parenthesis to indicate that they are rougher approximations than the others. This difficulty tends to make the count too low.

The uniformity of counts made under similar temperature conditions is such as to warrant the conclusion that the difference correlated with change of temperature indicates a real change of rhythm.

TABLE 2

| | KILOS | COLD | | NORMAL | | HOT | |
|---|---|---|---|---|---|---|---|
| | | Primary | Total | Primary | Total | Primary | Total |
| Experiment 6; Subject F..... | 20 | 36 | 46 | 41 | 62 | 45 | 70 |
| | 36 | 30 | 46* | 43 | 62 | 47 | 67 |
| | 40 | | | | | 43 | 68 |
| Experiment 7; Subject R..... | 32 | 33 | 53 | | | | |
| | 42 | | | | | 50 | 63 |
| | 51 | | | 47 | 63 | | |

* This count was made on the record reproduced in figure 3B in which a shift in the zero current made the record overlap the time line. On this account two or three of the minor oscillations may have been missed in the count.

The forearm flexors, although extending to a greater depth below the skin and nearer to the large arteries, and consequently less easily chilled through, are in other respects more favorable for study than the smaller muscle of the hand. For, in the first place, the excursions are larger and more regularly rhythmical, and are therefore more easily counted. In the second place, it is possible to grade the intensity of contraction by means of a dynamometer. The subject gripped the instrument and, watching the pointer, maintained a steady contraction of any desired force. Thus we could compare records at different temperatures but identical strength of contraction.

Table 2 shows the results of two experiments with the forearm flexors, in the first of which the identity of strength of contraction was closely maintained.

The constancy of rhythm in a given experiment at a given temperature irrespective of the strength of contraction is strikingly illustrated here, thus supporting the statement to this effect already made by Piper.[21] This constancy serves also to emphasize the change of rhythm which occurs in this muscle group, as well as in the interosseous, with change of temperature.

Another method of estimating the action current frequency in the records taken from the forearm flexors was rendered possible by the regular rhythmicity appearing in portions of these records (see figs. 3 and 4). A large number of the most regular of these oscillations were selected and the time interval between their summits determined by careful measurement on the record. The average of those measured was then computed, and from this the average frequency of oscillations per second for the most rhythmical portions was estimated. By this method the following results were obtained

Experiment 6.....Cold 36, Normal 49, Hot 50
Experiment 7.....Cold 39, Normal 51, Hot 54

Here again the figures show an unmistakable correlation between temperature and frequency.

It is to be noted that in all of the figures given, the difference in frequency is far less marked between normal and hot than between normal and cold, and in some instances of the former no difference at all is revealed by the figures. This is to be expected inasmuch as the range of temperature above normal which is tolerable to the skin is far smaller than the range below normal. Especially is it probable in the case of the forearm flexors, to which the blood stream comes more directly from the large arteries, that normal temperature is too quickly re-established after withdrawal from hot water to render possible the recording of an increase in frequency with any certainty. The fact that the frequency was in no case lower and in most cases higher after heat than normal, helps to support the general conclusion which rests in the main on the comparison of normal with cold.

Although the figures we have given are of doubtful value as far as establishing a quantitative estimate of the changes, we feel that inspection of the records, fair samples of which are reproduced in figures 1 to 4, will leave no doubt that cooling the muscle slows the rhythm. And since we have no way of determining the actual fall of temperature

[21] Piper: Arch. f. Physiol., 1910, 213.

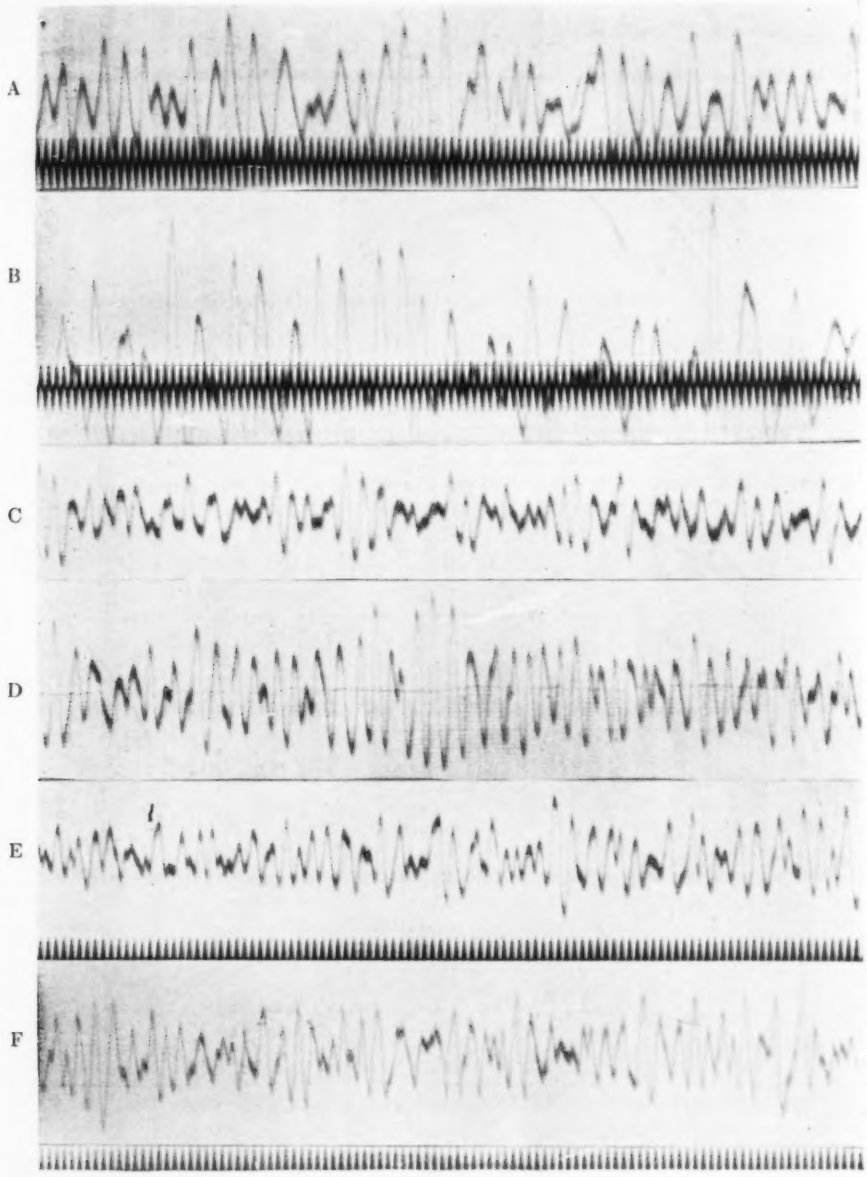


Fig. 3. Experiment 6; Subject F. Forearm flexors. *A* and *B* cold, after nineteen minutes' (interrupted) immersion, 4° to 9°C. *C* and *D* normal temperature. *E* and *F* hot, eight minutes' immersion 44° to 46°C. Strength of contraction in *A*, *C* and *E*, 20 kilos; in *B*, *D* and *F*, 36 kilos.

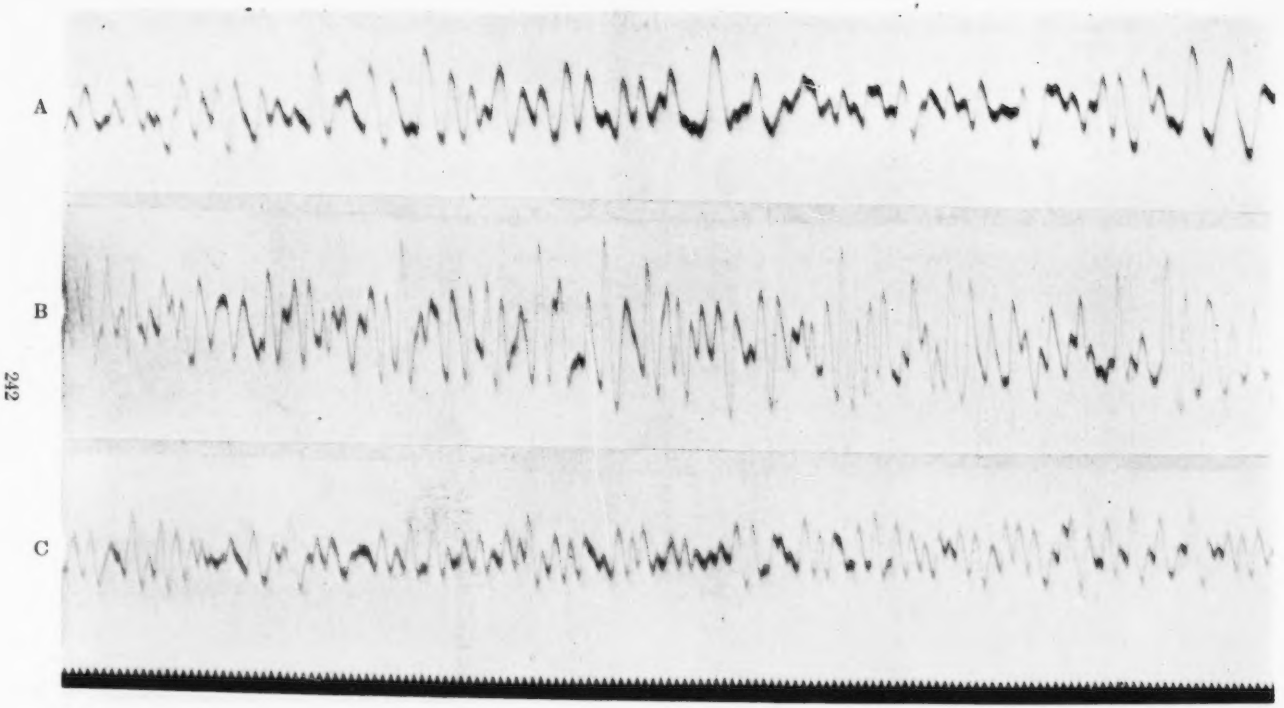


Fig. 4. Experiment 7; Subject R. Forearm flexors. *A*, cold, immersion fifteen minutes, 6°C., contraction 32 kilos; *B*, normal temperature, contraction 51 kilos; *C*, hot, immersion six minutes, 45° to 46°C., contraction 42 kilos.

in the muscles, an exact determination of the decrease in frequency would be of little value. The important fact is that an unmistakable decrease in frequency does occur when the muscle is cooled.

## DISCUSSION

It seems to us to follow from the above results that the rhythm of action currents appearing in the electromyogram of human voluntary contraction is no direct index of the rhythm of central innervation involved in the act. The change of rhythm attending change of temperature would be inexplicable on any such basis. For why should a change in the temperature of the muscle cause a change in the frequency of discharge of impulses from the ganglion cells whose temperature remains constant? This might conceivably be if afferent impulses coming from the chilled muscle so modified the nerve center as to alter its frequency of discharge; but this assumption is far-fetched and invokes nervous influences to which we know of no analogy. And since all the known phenomena, so far as we are aware, can be explained in terms of definitely known properties of nerve and muscle, without any such speculative assumption, it seems unnecessary to attach much weight to it. We agree, then, with Buchanan that the rhythm in the muscle does not follow the rhythm of motor nerve impulses, but depends rather on the condition of the muscle itself.

We now come to the task of reconciling this view with the fact pointed out by Piper and Beritoff,[22] that a frog's muscle can follow with separate action currents the rhythm of nerve impulses excited by induction shocks even up to a frequency of 250 or 300 per second. In man we have a higher possible frequency to reckon with, for V. Stern[23] has found by auscultating a muscle in a rabbit that a limiting frequency of 365 per second is possible in the mammalian muscle under like conditions of stimulation; and even without this observation we should expect a higher frequency under the temperature conditions prevailing in man than in the nerve-muscle preparation of the laboratory. We have here an apparent paradox; the muscle can respond separately to more than 300 nerve impulses per second, but when played on by the stream of impulses coming from the ganglion cells it responds with frequencies which may be evenly graded from 30 to 50 or more per second according to its own temperature. Sources of stimulation

[22] Piper: Arch. f. Physiol., 1910, 209; Beritoff: Loc. cit., 170.

[23] Stern: Pflüger's Arch., 1900, lxxxii, 39 (see Beritoff: Loc. cit., 171).

must be available at such frequency that the muscle can be excited at several frequencies between 30 and 50 per second. This requires the existence of such sources of stimulation at a frequency probably not less than 300 or 400 a second.

We are confronted here by the uncertainty as to whether we are dealing with propagated disturbances sweeping over the muscle "in a volley," i.e., simultaneously in all the fibers, or whether different groups of fibers respond in alternation or rotation.[24] In the latter case the rhythm of the individual fiber would be much slower than the rhythm appearing in the record. Piper's careful analysis[25] of the waves in his electromyograms, based on a study of artificially induced isolated waves, leads to the view that the rhythm appearing in the major oscillations is the actual rhythm of response in the individual muscle fiber. The fact that the frequency does not change with increased strength of contraction is in favor of this view. Inspection of a typical record suggests that where the curve is irregular it is due to certain fiber groups getting "out of step;" but where the oscillations are large and smoothly rhythmical, each double vibration closely resembling in size and time relations the record of a single response of the whole muscle,[26] it is highly probable that the majority at least of the fibers are responding in unison. If this is so, then the least number of nerve impulses in a given time would exceed the least common multiple of the various possible numbers of muscular responses which could, by changing the temperature, be made to occur in the same time with perfectly regular rhythm. Individual time measurements of the most regular oscillations in our records have been examined with a view to detecting any grouping which would on this basis suggest a nerve rhythm. Although it is possible to find practically all gradations in the intervals, yet with one subject (experiment 6) they seemed to be distinctly grouped at multiples of an interval which would indicate a nerve impulse frequency of 390 per second; with the other subject (experiment 7) a less distinct grouping was found such as would indicate a nerve impulse frequency of 260 per second. The intermediate values, espeally in the latter case, are sufficiently numerous to render a higher frequency than the last mentioned more probable. A conservative estimate would probably place 300 per second as the lower limit to the frequency of voluntary innervation. If this be the case, why does not

[24] Cf. Barbour and Stiles: Amer. Phys. Education Rev., Feb., 1912.

[25] Piper: Pflüger's Arch., 1909, cxxix, 150–168.

[26] See Piper: Elektrophysiologie, etc., 97, figs. 33 and 34.

the muscle respond at the maximum frequency possible, which Beritoff has found to be 250 per second in frog's muscle and is probably higher in human muscle?

The answer to this question may be found by examining the properties of nerve and muscle demonstrated by Lucas and Adrian.[27] In the first place, in considering the nature of the central stimulus, while we agree with Buchanan[28] that we have in these experiments no proof that it is discontinuous, we have good evidence from other sources that it is discontinuous or rhythmic and not in the nature of a steady flow of energy. Garten[29] has shown that stimulation with a constant current produces rhythmic electrical responses in a nerve. Moreover, the well established property of refractory period in nerve necessitates intermittent response. We must, then, regard the central stimulus as a series of separate impulses of unknown frequency; and our evidence seems to show that this cannot be less than 300 to 400 a second.

It is also well established that the refractory period in nerve and muscle has a "relative" as well as an "absolute" stage. Following the period in which no stimulus will excite there is a time during which the threshold falls gradually from infinity to its normal value.[30] During this "relative refractory period" of lowered excitability the magnitude of response is also subnormal. This has been shown by two wholly distinct criteria. First, Lucas[31] has shown that in nerve the action current resulting from a stimulus applied during this time is smaller than normal. Second, Lucas and Adrian[32] have both shown that a nerve impulse evoked during the relative refractory period is extinguished in a region of impaired conductivity through which a full-sized disturbance would pass. The recovery of excitability and the recovery of the magnitude of obtainable response proceed together during this time. Adrian[33] has also shown that at all times the magnitude of response in the conducting unit of nerve depends only on the local condition of the tissue, including under this head the stage of recovery during the relative refractory period, and is independent of the strength of stimulus.

[27] See references on p. 231, footnote 14; also Lucas, Proc. Roy. Soc., 1912, 85B, 495; and Adrian: Journ. Physiol., 1914, xlvii, 460.

[28] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 231.

[29] Garten: Loc. cit., 557.

[30] Adrian and Lucas: Journ. Physiol., 1912, xliv, 114.

[31] Lucas: Journ. Physiol., 1911, xliii, 51 and 77.

[32] Lucas: Loc. cit., 74; Adrian: Journ. Physiol., 1913, xlvi, 384.

[33] Adrian: Journ. Physiol., 1913, xlvi, 412.

With these facts in mind we may consider the question why the muscle exhibits in voluntary contraction only 50 responses per second when it is capable of 300 responses per second and is subjected to a still larger number of nerve impulses. The properties of these tissues just mentioned reveal to us the error in assuming for each tissue a "specific rhythm." For if a tissue be subjected to a continuous stimulus, the frequency with which it responds will depend on the intensity of the stimulus, for on this will depend the stage of recovery from one response at which the stimulus will become adequate and produce the next. As has already been pointed out by one of us[34] this variability of rhythm is well illustrated in the familiar class-room experiment on the "stannius heart," and the application of the principle to nerve has been well stated by Adrian.[35]

On this principle let us analyse the course of events in the neuromuscular system. Adrian and Lucas[36] found for frog's nerve at 15°C. an absolute refractory period of about $2\sigma$ and a total refractory period (including the "relative stage") of about $12\sigma$. For frog's muscle[37] (probably at a similar temperature) they found an absolute refractory period of about $10\sigma$ and a total refractory period of about $70\sigma$. They cite Bazett[38] as having shown in muscle a change of refractory period in a ratio of about 1:3 for a temperature change of 10°C. If the times are of the same order in human and in amphibian tissues, we may expect an 8- or 10-fold reduction of the refractory periods given above, in human tissues. Thus the relative refractory period for nerve would last roughly from $0.25\sigma$ to $1.5\sigma$ after an effective stimulus, and for muscle from about $1.2\sigma$ to $10\sigma$. The observations of Garten[39] on the mammalian nerve and muscle make it probable that these figures are too small. For the purposes of our analysis let us assume that the relative refractory period in human nerve begins $0.8\sigma$ and ends $7\sigma$ after the beginning of a previous response, and that in muscle it begins $3\sigma$ and ends $25\sigma$ after an effective stimulus.

If the nerve impulse when of subnormal magnitude serves as a weaker stimulus to the muscle fiber than it does when of normal magnitude, we have all the conditions necessary for a satisfactory explanation

[34] Forbes and Gregg: This Journal, 1915, xxxix, 231.
[35] Adrian: Loc. cit., 385–386.
[36] Adrian and Lucas: Loc. cit., 114.
[37] Loc. cit., 89.
[38] Bazett: Journ. Physiol., 1908, xxxvi, 426.
[39] Garten: Loc. cit., 543–552.

of the facts. Let us consider the stimulating value of the nerve impulse to the muscle as represented in an arbitrary scale of units in which we can also express quantitatively the threshold of the muscle. We can then plot a curve representing the magnitude the nerve impulse would have if evoked at each instant during the relative refractory period, i.e., during the recovery of the nerve to normal; and the magnitude will be expressed in terms of its stimulating value to the muscle. On the same system of coordinates we can plot a second curve representing the return of the threshold of the muscle fiber from infinity to normal during its relative refractory period; and the threshold will be expressed in terms of the requisite stimulating value of the nerve impulse. The

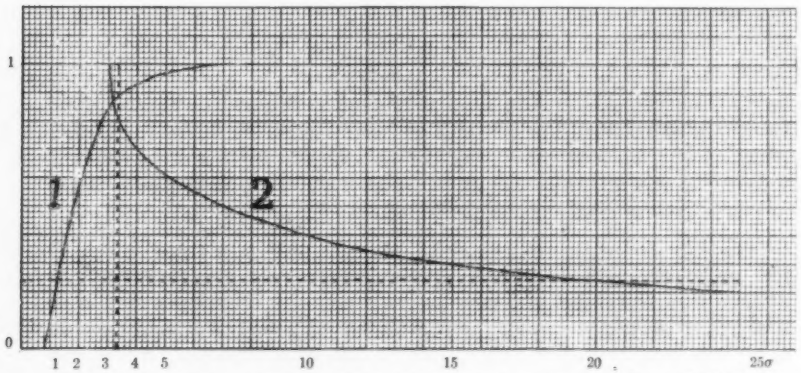


Fig. 5. Abscissae, time in $\sigma$ since previous adequate stimulus. Ordinates, stimulating value of nerve impulse (curve 1), threshold of muscle (curve 2), see text.

curve of nerve recovery will have in a general way the shape indicated in curve 1, figure 5; the curve of muscle threshold will be in a general way like curve 2.

We can now examine the course of events when the muscle is made to respond 300 times a second to as many induction shocks applied to the motor nerve, which Beritoff and Piper have both shown is possible. Each stimulus comes $3.3\sigma$ after its predecessor and each nerve impulse after the first will have the magnitude indicated on curve 1 at this point of time. If the muscle has responded to the previous stimulus its threshold will have at this instant the value indicated on curve 2. If the value indicated by curve 1 is higher at this instant than the

value indicated by curve 2 the muscle will be again excited. In the particular case represented by these curves it is clear that the muscle will be excited again at the stated time $3.3\sigma$ after the previous stimulus. The limit of frequency with which the muscle can respond to stimulation through its nerve depends on the time at which the curves cross. The validity of the principle invoked does not depend on the accuracy of these particular curves in expressing all the quantities. The procedure is merely a method of schematically representing established principles which determine the maximum frequency of response in a muscle stimulated through its nerve.

The next step is to trace the course of events in sustained voluntary contraction of muscle. Here, if our reasoning is correct, we are dealing with nerve impulses of a higher frequency than those just considered; each impulse occurs earlier in the relative refractory period following its predecessor and is consequently smaller and acts as a weaker stimulus to the muscle. After the muscle has once responded its threshold returns to normal so slowly that if the nerve impulses follow each other close enough several may pass before another suffices to excite the muscle; for the muscular threshold remains above the stimulating value of the weaker impulses throughout a larger part of its relative refractory period than in the case of the less frequent and stronger impulses. In the particular case illustrated by the curves it is only necessary to assume that the nerve impulses have a frequency of 800 per second in order that we may have all the conditions for a 50 per second rhythm in the muscle. This may be seen by reference to figure 5. Every nerve impulse following its predecessor at an interval of $1.25\sigma$ (i.e., 1/800 sec.) has the stimulating value required to excite the muscle $20\sigma$ after a preceding response, as is indicated in the figure by the horizontal dotted line. All the nerve impulses falling in the earlier part of the muscle's refractory period are ineffective.

Again we must note that the validity of this analysis does not rest on the correctness of the quantities we have assumed. The rhythm of 800 per second happens to be that which, according to the curves as we have drawn them, would condition a muscle rhythm of 50 per second. If the curves of recovery for nerve and muscle could be accurately determined and plotted, the requisite nerve impulse frequency might prove quite different; but the principle would apply just the same.

Thus is explained how a higher frequency of nerve impulses can cause a lower frequency of muscle responses. The application of this principle has been experimentally demonstrated by both Beritoff and

Buchanan. Beritoff[40] found that whereas the muscle kept pace with the rhythm of nerve stimulation up to 250 per second; with higher frequency of stimulation (300 per second) the frequency of muscular response fell to 150 per second, and in portions of the record, to 100 per second. Buchanan[41] found that stimulation of a motor nerve or of a curarized muscle with frequencies of 1000 per second produced in the muscle frequencies of response between 50 and 100 per second. When the frequency of stimuli was increased above 1000 no change occurred in the resulting muscle rhythm, and it is probable that these stimuli were virtually continuous even to the nerve, i.e., that the nerve was unable to follow the rhythm of stimulation, but responded in a rhythm dependent on its own refractory period.

We are now in a position to reinforce our previous argument for a high frequency of nerve impulses. We showed experimentally that the muscular rhythm of 50 per second was not the rhythm of the motor nerve, and that judging from the frequencies obtainable in muscle, the nerve rhythm must be much more rapid than this, probably not less than 300 per second. Now in the light of our analysis we can state that the nerve frequency cannot be less than 300 per second, for if it were the muscle would be able to follow it; and this it obviously does not.

Another fact noted by Beritoff,[42] harmonizing with the view here set forth, is that when the frequency of nerve stimulation is raised above 100 per second the muscle rhythm fails to follow regularly unless the stimuli are somewhat above threshold value. At this frequency he was already encroaching on the relative refractory period of the nerve, which under like conditions Adrian and Lucas[43] found to end about $12\sigma$ after the previous stimulus. With frequencies between 200 and 250 per second much stronger stimuli were needed to make the muscle follow the rhythm imposed, for now each stimulus found the excitability of the nerve far below normal. Buchanan[44] reports a similar observation. V. Stern[45] also found on ausculation that in the rabbit with stimuli of more than the critical frequency of 365 per second the tone fell to an octave below the stimulation pitch if the stimuli were strong, but with weak stimuli it fell two or even three octaves.

[40] Loc. cit., 170, fig. 26.
[41] Buchanan: Journ. Physiol., 1901, xxvii, 125.
[42] Loc. cit., 166.
[43] Loc. cit., 114.
[44] Buchanan: Quart. Journ. Exper. Physiol., 1908, i, 236.
[45] Loc. cit.

One important conclusion should be drawn from this discussion, that it is not permissible to assume that any tissue has one "specific rhythm" peculiar to itself.[46] The rhythm or response obtainable from a tissue is a resultant of the curve of recovering excitability and the strength of stimuli employed. In the case of muscle excited through its nerve we have two refractory periods to consider, that of the muscle itself and that of the nerve, and also the lowered stimulating value of the impulse traversing the nerve during its relative refractory period. Thus in the case of voluntary contraction we cannot say that the rhythm of muscular response is wholly of peripheral origin because it depends on peripheral conditions, for it depends also on central conditions and is therefore of compound origin, a resultant of several causes.

Piper[47] in support of his view that the rhythm of central innervation is 50 per second has presented the following evidence. Although the muscular rhythm is constant when the strength of contraction is changed, yet it is possible to reduce the frequency of recorded waves to one-half "by continuing a strong voluntary contraction to the point of complete fatigue." This, he argues, is to be explained by the failure of the fatigued central nervous system to send out the usual number of impulses. This interpretation follows, in his view, from the fact that the individual action currents recorded still show their normal amplitude and duration, therefore the muscle is not fatigued and the change must be associated with the nerve center.

These facts are quite as easily explained in another way which does not conflict with the evidence pointing to a higher nerve impulse frequency. Lucas[48] has shown that in a fatigued nerve-muscle preparation the resistance to the passage of a propagated disturbance increases at the neuromuscular junction. The result of this in the case of voluntary contraction, maintained, as we contend, by subnormal nerve impulses of high frequency, would be to cut down still further the stimulating value of the impulses at the point where they act on the muscle fibers, and consequently to delay until nearer the end of their relative refractory period the time at which they would respond. Thus, without change in the nerve impulse frequency, the mere raising of resistance in the neuromuscular junction would slow down the rhythm of muscular response.

[46] See Garten: Loc. cit., 535.
[47] Piper: Arch. f. Physiol., 1910, 214.
[48] Lucas: Journ. Physiol., 1911, xliii, 76.

The fact shown by Piper and confirmed in our records that the rhythm of voluntary muscular response in absence of fatigue is independent of the strength of contraction brings up an interesting question as to the mechanism of gradation. One of us[49] in a previous paper has proposed as a basis of sensory gradation the rhythm of afferent impulses, which could easily be conditioned by the intensity of peripheral stimulation on the same principle of relative refractory period which we have just been considering. That a similar gradation of muscular contraction can depend on the frequency of muscular response seems to be disproved by the above observations. So far as we can see, unless we reject the "all-or-none" view of muscle contraction, so well supported by Lucas,[50] we are forced to the view that in voluntary contraction gradation must be conditioned by gradation in the number of muscle fibers in action at a given moment, the doctrine of fractional activity, as Stiles[51] has termed it.

It would be interesting to form an estimate, if possible, of the actual frequency of nerve impulses involved in voluntary muscular contractions. We have furnished evidence which indicates that it is probably not less than 300 or 400 per second. We have shown that from the refractory periods measured in frog's nerves and the temperature coefficient determined for them, we can infer that the absolute refractory period in mammalian nerves at their normal temperature may be as short as $0.2\sigma$, which would make possible an extreme frequency of 5000 per second. Between these limits we have little to guide us in estimating the probable frequency. Garten,[52] stimulating mammalian nerves at their normal temperature with a constant current, obtained action currents showing frequencies between 300 and 500 per second. These values do not necessarily show the highest possible frequency, since the intensity of the stimulus may have been so low as only to excite the nerve toward the end of its relative refractory period.

Whatever the frequency of impulses in the motor nerve under natural conditions of central discharge, it probably bears some relation to the refractory period of the nerve fiber. We feel that this consideration tends in a way to support the general conclusion that the nerve impulse frequency is much higher than that of the action currents

[49] Forbes and Gregg: This Journal, 1915, xxxix, 229–232.

[50] Lucas: Journ. Physiol., 1905, xxxiii, 125; 1909, xxxviii, 113.

[51] Stiles: Amer. Phys. Education Rev., 1910, xv, 1; Barbour and Stiles: Loc. cit.

[52] Garten: Loc. cit., 546 and 554.

recorded in the muscle. Otherwise the wonderfully short refractory period of nerve would appear a meaningless and useless property. That a nerve fiber can recover its ability to respond with such great rapidity is one of its most remarkable properties and one which perhaps more than any other marks its high development in the evolutionary process. To suppose, then, that in the ordinary performance of its function this ability should be wasted, and that although capable of transmitting several hundred impulses per second the nerve fiber is only called on to transmit fifty, implies a lack of economy quite out of keeping with the usual degree of adaptation found in the functions of living tissues. It might be argued that the high frequency of nerve impulses is wasted if the muscle only responds to a small proportion of these; but a large excess of available impulses may serve a useful purpose by providing in some way a greater variability in the timing and coordination of muscular response.

In a previous paper one of us[53] has mentioned reasons for suspecting that the central elements involved in reflexes may have much shorter refractory periods than even the nerve fibers. Should this prove to be a general and essential property of the central nervous mechanism, it might be that the activity of this mechanism whenever involved in the causation of sustained muscular contraction is even more rapidly rhythmical than the impulses in the motor nerve fiber. This mechanism may bear the same relation to the nerve fiber that the nerve fiber does to the muscle, in that it supplies to the nerve fiber a series of stimuli of such high frequency that they amount to a continuous stimulus as far as the nerve fiber is concerned, and the nerve fiber responds with a frequency determined by its own relative refractory period. But this suggestion is too highly speculative to merit more than passing mention.

The "waves" which Buchanan[54] has found in her records of strychnine spasm in the decerebrate frog, occurring with frequencies between 3 and 14 per second, and which she showed to be associated with the condition of the nerve centers, obviously cannot be regarded as indicating the frequency of individual nerve impulses. Such slow rhythms as these should be classed, together with other slow rhythms such as are seen in clonus, the scratch reflex and others noted myographically,[55]

[53] Forbes and Gregg: Loc. cit., 221.

[54] Buchanan: Journ. Physiol., 1901, xxvii, Plates vii, viii and ix; also Quart. Journ. Exper. Physiol., 1908, i, 215, etc.

[55] See Sherrington: Quart. Journ. Exper. Physiol., 1913, vi, 284; Proc. Roy. Soc., 1913, 86B, 233; Graham Brown: Proc. Roy. Soc., 1912, 85B, 278; Forbes: Proc. Roy. Soc., 1912, 85B, 289.

as group rhythms. Each beat in such a rhythm must represent a large group or series of nerve impulses discharged from the center at a far higher frequency, and followed by a lull or cessation of activity. Many such rhythmic tendencies are evident in the functioning of nerve centers, but their essential nature has yet to be explained.

One striking feature of our records, which has already been mentioned, is the increased amplitude in the galvanometer excursions on cooling the muscle, and their decreased amplitude on heating. This is in part due to the lag of the string, which being of platinum has considerable inertia. This lag or failure of the string to follow electromotive changes with precision, distorts the curve more in the case of rapid oscillations than in the case of slow ones. The result is a slight apparent decrease in magnitude as the oscillations become more rapid. But the change in action current frequency is so small and the change in amplitude of the excursions so great that we feel little doubt that there is a true increase in the strength of individual action currents when the muscle is cooled.

In accordance with our interpretation of the phenomena such an increase in the magnitude of individual responses might be explained in one of two ways. One explanation depends on the conceivable separability of excitability and magnitude of electrical response in the process of recovery from the refractory period. If in a cool muscle the excitability is longer delayed in its return to normal than is the possible magnitude of action current resulting from an adequate second stimulus, then the action currents, under such conditions of nerve stimulation as we have pictured, will be increased in magnitude. For the stimuli delivered by the nerve will not be effective on account of the lowered excitability, until a stage of recovery at which the magnitude of response will surpass that found at the higher temperature.

Such an explanation is most unlikely since Adrian[56] has shown that in the case of nerve the possible magnitude of disturbance, as judged by the functional criterion of its ability to pass through a region in which it undergoes a decrement, recovers simultaneously with excitability, after the passage of a previous disturbance. That the action current is a true measure of the functional magnitude of a propagated disturbance is not proved, but all evidence points strongly to that view.[57] Again, Adrian proved his point for nerve and not for muscle, but for muscle to differ from nerve in the separability of excitability from

[56] Adrian: Journ. Physiol., 1916, l, 345.

[57] Forbes, McIntosh and Sefton: This Journal, 1916, xl, 503.

magnitude of response in the recovery process would imply a fundamental difference between the tissues with respect to a property in which all evidence points to their essential similarity.[58]

Another explanation which invokes no such improbable property in muscle as the separation of excitability and magnitude of response in the recovery process, depends on the cooling of the distal portions of the nerve fibers which innervate the cooled muscle. Impulses traversing these fibers in their relative refractory period are subnormal, and on passing from a warm to a cool portion of the nerve they enter a region where the refractory period is prolonged, and are still further reduced in magnitude. From this it follows that they will not excite the muscle till still later in its relative refractory period than would be the case if the nerve fibers were warm throughout their course. The muscle excited later in its recovery stage, will yield a larger response. Thus may readily be explained the increased magnitude of action currents in the cooled muscle without the need of any improbable assumption.

## SUMMARY

1. By local immersion in cold water it is possible to reduce the frequency of the rhythmic action currents in the muscles of the hand or forearm during voluntary contraction, while the body temperature, recorded by mouth, remains constant. Immersion in hot water makes the rhythm more rapid, but the change is less marked than in the case of cold water, as is to be expected from the fact that a bigger change of temperature is possible below than above normal.

2. This evidence shows that the rhythm observed in muscle response (usually about 50 per second) is not the rhythm of motor nerve impulses, but is dependent, as Buchanan maintains, on the condition of the muscle.

3. A careful study of the possible gradations of muscular rhythm, together with a consideration of other known facts concerning the refractory period in nerve, leads to the conclusion that the actual frequency of motor nerve impulses involved in evoking voluntary contraction must lie somewhere between 300 and 5000 per second.

4. Previous objections to the view that the nerve impulse frequency is higher than that appearing in the muscular response are answered by an analysis of the relation between the "relative refractory periods" of nerve and muscle.

[58] See Lucas: Proc. Roy. Soc., 1912, 85B, 495.

5. We have confirmed Piper's observation that the action current rhythm in muscle is independent of strength of contraction.

6. Besides the decrease in frequency following a fall of temperature there is an increase in amplitude of galvanometric excursions, denoting an increase in the strength of the individual action currents. This can most easily be explained as a result of the cooling of the distal portions of the nerve fibers, the explanation depending on the various factors involved in the relative refractory periods of the nerve and muscle fibers.

# BILE PIGMENT METABOLISM

## III. Bile Pigment Output and Blood Feeding

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When it was shown that the bile pigment secretion in the dog can be influenced at will by changes in diet, it became imperative to try various substances rich in the pyrrol nucleus. This paper deals with the influence of blood feeding on the bile pigment elimination in the dog.

In earlier publications we have shown that carbohydrates tend to increase considerably the secretion of bile pigments in dogs with bile fistulae (1). On a strict meat diet the bile pigment curve is at its lowest level, but with a sharp transition to a diet rich in carbohydrates, a rise of 50 to 100 per cent in bile pigment output may be noted. We believe sufficient experimental data are at hand to prove this point beyond a doubt, but the explanation of this peculiar and important reaction is not clear. We hope to report experiments in the near future which will throw some light on this important point.

It is very tempting to assume that the liver can construct certain pigments regardless of more or less blood destruction or that it can assemble more or less completely some parent pigment complex which may be used for one purpose or another as needed in the body economy. This is a stimulating possibility to be kept in mind, and is responsible for much of this work to be reported. Whether this hypothesis can be established beyond a reasonable doubt remains to be seen.

Our first paper (2) reviewed the work of other investigators in this field, and pointed out some of the many difficulties met with in work with these bile fistula dogs. It was shown that under very uniform conditions of health and routine collection the output of bile pigments becomes quite constant for days, weeks and months. The mean output per six hour period is 1 mgm. per pound body weight, or very

slightly below this. There are hourly and at times daily variations which can not be explained in the light of our present knowledge. For this reason experiments must be repeated time and again with extremely careful control observations. This point cannot be too strongly emphasized, as has been done by Stadelmann (3).

The various experimental procedures employed in this work have been carefully described by us in earlier papers (2), and will not be reviewed here. A new method which is rapid, simple and accurate for the quantitative estimation of bile pigments in whole bile was described in detail. There are no departures from these published methods, unless note is made to that effect. It will be noted that several of the bile fistula dogs are marked with a splenectomy. There are many interesting points to come up for presentation in another paper dealing with splenectomy and bile fistulae, but in these feeding experiments the absence of the spleen has no effect.

TABLE A

*Fresh sheep blood feeding and bile pigment secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | Weight | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 15 | 35 | 27 | 30 | | 92 | 13.3 | 8.2 | 10.7 | | 32.2 | 31.0 | |
| March 16 | 32 | 33* | 23 | 27 | 83 | 11.6 | 13.4* | 9.4 | 10.3 | 33.1 | 31.5 | 300 cc. sheep blood |
| March 17 | 33 | 32 | 27 | | 92 | 12.0 | 10.8 | 9.1 | | 31.9 | 30.8 | |
| March 18 | | | | | 52 | | | | | 32.8 | 31.5 | |
| March 20 | 25 | 24 | 31 | | 80 | 8.9 | 9.6 | 10.4 | | 28.9 | 31.5 | Hemoglobin 116 per cent |
| Dog 15–27. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 7 | 18 | 23 | 23 | | 64 | 8.4 | 9.4 | 7.9 | | 25.7 | 25.5 | Hemoglobin 82 per cent |
| March 8 | 17 | 17 | 22 | | 56 | 6.5 | 7.6 | 8.4 | | 22.5 | 26.0 | |
| March 9 | 19 | 26* | 24 | 19 | 69 | 7.7 | 8.2* | 8.1 | 6.8 | 23.1 | 26.0 | 500 cc. sheep blood |
| March 10 | 9 | 17 | 24 | | 50 | 7.3 | 7.6 | 7.6 | | 22.5 | 26.3 | |
| March 11 | | | | | 42 | | | | | 20.8 | 26.5 | |

* Fresh sheep blood given through stomach tube at the beginning of the third hour.

TABLE B

*Fresh pig blood feeding and bile pigment secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 24 | 33 | 35 | 21 | | 89 | 12.0 | 11.0 | 6.2 | | 29.2 | 31.5 | |
| March 25 | | | | | 94 | | | | | 28.8 | 32.0 | Hemoglobin 119 per cent |
| March 27 | 22 | 26 | 25 | | 73 | 10.4 | 11.6 | 8.5 | | 30.5 | 31.5 | |
| March 28 | 34 | 26* | 26 | 25 | 77 | 10.0 | 7.6* | 10.5 | 9.0 | 27.1 | 31.5 | 350 cc. pig blood |
| March 29 | 26 | 29 | 29 | | 84 | 7.6 | 10.4 | 9.1 | | 27.1 | 32.3 | Stool contains stercobilin |
| Dog 16-10. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 24 | 29 | 36 | 21 | | 86 | 12.4 | 15.8 | 10.9 | | 39.1 | 32.0 | Stools contain stercobilin |
| March 25 | | | | | 56 | | | | | 37.6 | 31.5 | |
| March 27 | 17 | 17 | 28 | | 62 | 13.0 | 8.8 | 15.2 | | 37.0 | 31.0 | Hemoglobin 97 per cent |
| March 28 | 20 | 21* | 26 | 28 | 75 | 14.1 | 12.2* | 13.1 | 14.0 | 39.3 | 31.5 | 350 cc. pig blood |
| March 29 | 22 | 31 | 25 | | 76 | 13.8 | 13.6 | 14.6 | | 42.0 | 32.0 | Stools contain no stercobilin |

* Fresh pig's blood given through a stomach tube at the beginning of the third hour.

TABLE C

*Fresh ox blood feeding and bile pigment secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1-2 hrs | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 8 | 25 | 28 | 28 | | 81 | 8.5 | 8.8 | 10 0 | | 27.3 | 30.8 | Hemoglobin 112 per cent |
| March 9 | 28 | 37* | 21 | 28 | 86 | 9.4 | 9.7* | 6.9 | 10.0 | 26.6 | 31.5 | 400 cc. ox blood |
| March 10 | 24 | 25 | 25 | | 74 | 9.8 | 9.0 | 8.5 | | 27.3 | 31.3 | |
| March 11 | | | | | 64 | | | | | 26.4 | 31.5 | |
| March 13 | 20 | 25 | 19 | | 64 | 9.9 | 10.8 | 7.6 | | 28.3 | 31.3 | Hemoglobin 114 per cent |
| Dog 16–41. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 10 | 13 | 15 | 30 | | 58 | 10.2 | 7.7 | 12.8 | | 30.7 | 37.0 | |
| March 11 | | | | | 58 | | | | | 32.8 | 37.3 | |
| March 13 | 17 | 27 | 31 | | 75 | 9.2 | 10.3 | 9.8 | | 29.3 | 37.0 | Hemoglobin 81 per cent |
| March 14 | 34 | 27* | 40 | 46 | 113 | 11.5 | 10.3* | 9.2 | 9.6 | 29.1 | 37.3 | 500 cc. ox blood |
| March 15 | 48 | 36 | 27 | | 111 | 10.5 | 11.2 | 11.5 | | 33.2 | 37.8 | |

* Fresh ox blood given through stomach tube at the beginning of the third hour.

TABLE D

*Fresh dog blood feeding and bile pigment secretion*

Dog 16–10. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 7 | 21 | 27 | 24 | | 72 | 11.3 | 12.2 | 9.2 | | 32.7 | 31.3 | Hemoglobin 90 per cent |
| March 8 | 25 | 22 | 22 | | 69 | 10.8 | 10.4 | 10.9 | | 32.1 | 31.8 | |
| March 9 | 21 | 17* | 18 | 20 | 55 | 16.5 | 13.0* | 13.8 | 13.6 | 40.4 | 32.0 | 500 cc. dog blood |
| March 10 | 23 | 21 | 25 | | 69 | 14.0 | 13.4 | 13.5 | | 40.9 | 31.8 | |
| March 11 | | | | | 46 | | | | | 36.2 | 31.8 | |
| Feb. 28 | 13 | 23 | 23 | | 59 | 10.2 | 10.4 | 10.4 | | 31.0 | 31.0 | Hemoglobin 88 per cent |
| Feb. 29 | 21 | 19* | 19 | 21 | 59 | 13.4 | 11.5* | 10.8 | 9.4 | 31.7 | 30.5 | 200 cc. dog blood |
| March 1 | 23 | 19 | 20 | | 62 | 15.8 | 12.8 | 13.6 | | 42.2 | 30.5 | |
| March 2 | 18 | 22 | 24 | | 64 | 4.5 | 15.2 | 14.0 | | 33.7 | 31.0 | |
| March 3 | 23 | 25 | 22 | | 70 | 15.8 | 14.6 | 15.4 | | 45.8 | 31.8 | |

* Fresh defibrinated dog blood given through stomach tube at the beginning of the third hour.

TABLE E

*Hemoglobin feeding and bile pigment secretion*

Dog 16–104. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 1 | 25 | 19 | 19 | | 63 | 10.2 | 8.9 | 8.9 | | 28.0 | 33.0 | |
| March 2 | 24 | 23* | 29 | 27 | 79 | 11.3 | 10.3* | 11.7 | 10.3 | 32.3 | 32.8 | 150 cc. laked red cells |
| March 3 | 23 | 23 | 18 | | 64 | 8.2 | 10.9 | 8.1 | | 27.2 | 32.8 | |
| March 4 | | | | | 82 | | | | | 29.4 | 33.0 | |

* 150 cc. washed dog's red blood cells laked with equal quantity of distilled water given through stomach tube at the beginning of the third hour.

TABLE F

*Cooked pig blood feeding and bile pigment secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 29 | 26 | 29 | 29 | | 84 | 7.6 | 10.4 | 9.1 | | 27.1 | 32.3 | |
| March 30 | 35 | 34* | 34 | 28 | 96 | 10.2 | 9.2* | 10.0 | 8.2 | 27.4 | 32.5 | 200 cc. cooked blood |
| March 31 | 25 | 36 | 31 | | 92 | 9.0 | 12.0 | 9.1 | | 30.1 | 32.5 | |
| April 1 | | | | | 48 | | | | | 25.8 | 32.8 | |
| April 3 | 22 | 21 | 31 | | 74 | 10.4 | 11.5 | 10.4 | | 32.3 | 32.0 | Hemoglobin 116 per cent |
| Dog 16–41. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 24 | 35 | 55 | 25 | | 115 | 11.0 | 14.1 | 7.3 | | 32.4 | 37.3 | |
| March 25 | | | | | 94 | | | | | 33.2 | 36.8 | |
| March 27 | 36 | 30 | 28 | | 94 | 14.4 | 13.6 | 10.6 | | 38.6 | 37.0 | Hemoglobin 93 per cent |
| March 28 | 19 | 18* | 30 | 26 | 74 | 14.4 | 14.4* | 12.8 | 11.6 | 38.8 | 36.8 | 350 cc. cooked blood |
| March 29 | 29 | 35 | 38 | | 102 | 9.2 | 11.0 | 10.3 | | 30.5 | 37.3 | |

* Cooked pig's blood given through stomach tube at the beginning of the third hour.

TABLE G

*Digested ox red cell feeding and bile pigment secretion*

Dog 16–10. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 29 | 22 | 31 | 23 | | 76 | 13.8 | 13.6 | 14.6 | | 42.0 | 32.0 | |
| March 30 | 19 | 21* | 27 | 29 | 77 | 14.6 | 14.4* | 14.6 | 12.4 | 41.4 | 32.5 | |
| March 31 | 19 | 23 | 25 | | 67 | 11.0 | 12.9 | 12.4 | | 36.3 | 32.3 | |
| April 1 | | | | | 48 | | | | | 37.8 | 32.3 | |
| April 3 | 19 | 20 | 28 | | 67 | 14.1 | 11.6 | 15.8 | | 41.5 | 30.8 | Hemoglobin 102 per cent |

* 100 cc. digested ox red blood cells given through stomach tube at the beginning of the third hour. Blood cells digested at 38°C. with pancreatin.

TABLE H

*Digested ox blood feeding and bile pigment secretion*

Dog 16–41. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 18 | | | | | 46 | | | | | 32.2 | 38.0 | |
| March 20 | 38 | 32 | 35 | | 105 | 12.0 | 10.8 | 10.2 | | 33.0 | 37.0 | Hemoglobin 94 per cent |
| March 21 | 20 | 37* | 37 | 40 | 114 | 10.8 | 11.3* | 7.3 | 7.2 | 25.8 | 37.3 | 100 cc. digested blood |
| March 22 | 46 | 33 | 40 | | 119 | 10.4 | 9.7 | 9.0 | | 29.1 | 38.0 | |
| March 29 | 29 | 35 | 38 | | 102 | 9.2 | 11.0 | 10.3 | | 30.5 | 37.3 | |
| March 30 | 28 | 25* | 40 | 37 | 102 | 10.6 | 9.0* | 11.6 | 9.7 | 30.3 | 37.0 | 150 cc. digested blood |
| March 31 | 21 | 24 | 28 | | 73 | 8.4 | 9.2 | 9.4 | | 27.0 | 37.3 | |
| April 1 | | | | | 46 | | | | | 31.6 | 37.3 | |
| April 3 | 41 | 22 | 28 | | 91 | 12.2 | 8.9 | 8.8 | | 29.9 | 36.3 | Hemoglobin 99 per cent |

* Digested whole ox blood given by stomach tube at the beginning of the third hour. The blood was digested for weeks with pancreatin at 38°C.

## EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments had been under observation following a uniform routine for months and some for over a year. They were all in good condition with constant body weight, normal blood and uniform bile pigment secretion. Unless otherwise noted, these dogs showed only a trace of bile pigment in the urine, no stercobilin in the feces and no urobilin in the urine. Under such conditions a small variation in bile pigment output is worth careful consideration, provided the observation is constant in repeated experiments.

It may seem that repeated experiments with blood of different animals are not called for. It should be noted in other papers to follow that bile from different animals may have a constant effect on total bile secretion in the dog when given by mouth, but that bile of one animal may stimulate and bile of another animal may inhibit the bile pigment secretion. For this reason we report the observation on blood feeding in the dog, using blood from different animals.

## DISCUSSION

The tables given above speak for themselves, and the results are quite uniform. Many more experiments have been done with this same material, and the results are identical. These other tables are not given for lack of space, but we feel that a sufficient number of experiments have been performed to make the point quite certain, and only a few type experiments are here recorded.

Blood feeding has no influence on the secretion of bile pigment in the normal dog. Whole blood of the sheep, pig or ox freshly defibrinated given to the dog by stomach tube does not influence the output of bile pigments nor the volume of bile. Freshly laked dog red cells given by stomach tube likewise have no effect on bile secretion. Cooked pig blood and digested ox blood have not the slightest influence on the output of bile pigments. There is no immediate effect nor any delayed influence on the bile secretion.

This diet is very rich in the pyrrol nucleus, perhaps contains it in as concentrated form as it occurs in normal body tissues or fluids. It seems safe to assume that during the period of observation a certain amount of this material was digested, and its constituent parts taken in through the intestinal mucosa. We propose to show that other substances in the diet besides carbohydrates will cause a rise in bile pigment output, but hemoglobin is not one of this group. It may be possible in the near future to indicate from what "building stones" the body constructs its various pigments which are so closely related. Perhaps the story may yet be written of how certain substances may be used when needed in pigment building or put aside in reserve as certain by-products or eliminated from the body in the form of one or another pigment.

## CONCLUSION

The flow of bile and the secretion of bile pigments in the dog are not influenced by the ingestion of fresh blood of the dog, sheep, pig or ox.

Freshly laked red cells by mouth likewise have no effect.

Cooked pig blood or digested ox blood given by stomach have no influence on the secretion of bile pigments.

These negative findings are of some importance in view of the fact that carbohydrates by mouth cause an increased secretion of bile pigments in dogs.

Splenectomy causes many interesting reactions in bile fistula dogs, but it does not influence the output of bile pigments under normal conditions. Splenectomy has no effect upon these blood feeding experiments.

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# BILE PIGMENT METABOLISM

## IV. Influence of Fresh Bile Feeding upon Whole Bile and Bile Pigment Secretion

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This paper presents the experimental data resulting from feeding fresh bile from various animals to dogs with bile fistulae. Bile feeding causes a very definite increase in bile flow from the fistula, as is well known, but also, with few exceptions, it causes a definite *diminution* of the outflow of bile pigments in spite of active cholagogue action, which might be expected to sweep an excess of bile pigments out of the liver. This sweeping out of an excess of bile pigments during cholagogue action is exactly what we might expect, provided the liver had simply an eliminative function in disposing of worn out corpuscles and hemoglobin by way of the bile. In the following paper the various constituents of bile are tested on bile fistula dogs, and the striking effects are analysed.

That bile feeding has a definite and striking effect on the bile pigment secretion is established, and is of considerable importance in understanding this obscure story of pigment metabolism. Can we assume that bile feeding causes a decrease in the continuous disintegration of red cells which we are told is going on with such rapidity in the body, and is solely responsible for the outpouring of bile pigment? There may be a reasonable doubt on this point. It is to be recalled that most of the evidence concerning the short life cycle of the red cell is based on end product determinations. If there is a suspicion that other substances than hemoglobin may be formed into these same end products or that some of the used up hemoglobin may not be formed into this same end product, we may also question the life cycle of the red cell as determined in this manner. At least we may say that there is a possibility of error, and assume that the life of a red cell *may* be

normally more than ten to fourteen days. This important point will be taken up in later communications.

It is proper at this point to take up the question of "bile circulation" as applied to the bile pigments. Wertheimer (1) injected sheep's bile into dogs' portal veins, and showed that cholohematin appeared promptly in the dog's bile, and could be recognized by its characteristic spectrum. Curiously enough many writers quote this work as evidence that the bile is absorbed from the *intestine*, and that there is a circulation of bile pigments as well as bile salts. Stadelmann (2) leaves the question open, and says that very small amounts of bile pigment *may* be absorbed from the intestine. All our experiments give not the slightest evidence of any bile pigment absorption from the intestine, and the evidence seems sufficient to rule out any such possibility in dogs. We feel safe in claiming that there is no "bile circulation" of the bile pigments as such.

Another type of pigment circulation has been suggested by Addis (3), and has a strong appeal because of its very simplicity and directness. He suggests that the following outline may cover much of the life history of hemoglobin pigment. Hemoglobin is constantly liberated in the body from the disintegration of worn out red cells. This hemoglobin is changed in the liver to bile pigments, and excreted into the intestine. The bile pigment is reduced to urobilinogen in the intestine, a part escaping in the feces, and an important part being absorbed into the blood, polymerized into urobilin-complex, and taken up by the liver. This portion is stored in the liver or synthesized into hemoglobin, or, if the liver is abnormal, may escape into the blood and appear in the urine as urobilinogen. This hypothesis in a word is "bile circulation" again presented in a more elaborate form—a circulation of a pyrrol complex.

A part of this cycle may be admitted as true, that the pigment radicle of hemoglobin may appear in the bile as bile pigment and later as stercobilin or urobilinogen in the intestine. Is there any evidence that urobilinogen is absorbed from the intestine into the blood? Not one grain of direct evidence, as urobilinogen has never been demonstrated in portal blood. It is claimed that the stercobilin of the feces is insufficient to account for all the bile poured into the duodenum, therefore, some of it must be absorbed. There are several unknowns in this equation. It is claimed that urobilin is formed only in the intestine, and must be absorbed from it, because persons with common duct obstruction do not show urobilinogen nor stercobilin. It is

also known that many other disorders result from exclusion of the bile from the intestine, and one could assume that such disturbances might react on other organs. It is possible to assume that urobilinogen may be produced at times in the liver (like other pigments), and that this production may depend in part on liver function (liver disease). There is not a bit of evidence produced so far which will not fit this hypothesis as well or better than the usual theory that urobilin is formed only in the intestine.

There is no direct evidence at hand to contradict us if we assume that urobilin under certain conditions may be produced outside of the intestine, perhaps in the liver or even kidney. With the evidence at hand to indicate that the production of one common body pigment (bilirubin) is possible outside of the liver (4), we must be guarded in our statements that some other common pigment (urobilin) may not be formed under a variety of conditions, particularly abnormal conditions.

Addis states that the importance of hemoglobin presupposes conservation by the body of this unusual pyrrol group. This may be granted as desirable for the sake of argument, but direct evidence of this must be brought forward before the hypothesis can be accepted. There is no direct evidence that urobilinogen is absorbed from the intestine, and the indirect evidence is capable of other interpretation.

There is strong indirect evidence against this intestinal absorption and "circulation" of the pyrrol group. Large doses of bile by mouth in bile fistula dogs cause *decrease* in bile pigment output. But much more important is the fact that dogs under observation for months and years with bile excluded from the intestine fail to develop an anemia. Such dogs over periods of months have been kept so that the feces were free from stercobilin, yet no anemia developed. This shows that the dog is in no way dependent for its supply of hemoglobin upon pigments absorbed from the intestine. These experiments do not exclude the possibility of any pigments being absorbed from the intestine, but they do show that if such absorption does take place it is of trivial importance in the pigment metabolism. The burden of proof must now rest with those who claim that there is some pigment absorption from the intestine in view of the fact that normal pigment metabolism goes on when all such absorption has been prevented over long intervals of time.

## EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments make up the same group used in the preceding paper. In all cases bile was absent from the urine or present at the most in traces. Stercobilin as a rule was absent unless a note is made to the contrary. The routine treatment and diet of the dogs have been described previously (5), also the methods of collection and analysis. The dogs were all in good condition, and were in no way upset by these various experimental procedures, so it is not necessary to give protocols except in the form of tables.

Tables 1 and 2 show a pretty constant and sometimes an extreme cholagogue action following the introduction of dog bile by the stomach tube. There is a striking fall in bile pigment secretion appearing after two or four hours or sometimes as promptly as the cholagogue action during the first two hours. This fall is all the more noticeable as it appears during a high bile output. The bile pigment secretion is normal by the next day, but sometimes the cholagogue action seems to persist into the second day. Splenectomy does not influence this reaction.

Tables 3, 4 and 5 show the variation which may be noted after the feeding of dog bile. We have many more experiments, but the ones submitted here are good examples of two main groups. One group (tables 1 and 2) show more or less cholagogue action and a definite depression of bile pigment output. The other group (tables 3, 4 and 5) shows the same cholagogue action but little depression of the bile pigment secretion. There are border line experiments between these two groups. The reason for this comes out in the next paper of this series. It will be seen in the above tables that even if the total pigment output is equal to the average of previous days, there is a tendency toward a falling curve of secretion (table 3) following the ingestion of bile.

Table 6 gives the results of two experiments of a group of four. The results in each experiment were identical. The bile feeding by stomach tube was given in the early morning, and the bile collection done nine hours later. It is clear that there was no influence upon the output of bile pigments—no delayed reaction. One dog shows a cholagogue action and the other is negative. These experiments are necessary to meet the possible objection that the inhibition of bile pigment secretion might be only temporary to be followed by a proportional increase within a few hours.

TABLE 1

*Dog bile feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 3 | 35 | 48 | 35 | | 118 | 12.8 | 10.2 | 11.8 | | 34.8 | 30.8 | Stools contain stercobilin |
| March 4 | | | | | 96 | | | | | 30.6 | 31.0 | |
| March 6 | 30 | 30 | 35 | | 95 | 8.8 | 10.0 | 9.4 | | 28.2 | 30.5 | |
| March 7 | 20 | 67* | 33 | 26 | 126 | 9.4 | 9.0* | 2.9 | 0.8 | 12.7 | 30.0 | |
| March 8 | 25 | 28 | 28 | | 81 | 8.5 | 8.8 | 10.0 | | 27.3 | 30.5 | Hemoglobin 112 per cent |
| April 12 | 15 | 23 | 27 | | 65 | 8.8 | 9.4 | 12.8 | | 31.0 | 31.0 | |
| April 13 | 17 | 25† | 32 | 34 | 91 | 10.6 | 10.2† | 3.6 | 2.3 | 16.1 | 30.5 | |
| April 14 | 18 | 29 | 29 | | 76 | 11.3 | 13.1 | 12.4 | | 36.8 | 30.8 | |
| April 15 | | | | | 42 | | | | | 32.0 | 31.0 | |
| April 17 | 28 | 24 | 24 | | 76 | 9.4 | 9.2 | 11.5 | | 30.1 | 31.0 | Hemoglobin 118 per cent |

* 250 cc. dog's bile containing 104 mgm. bile pigments given through stomach tube at beginning of third hour.

† 200 cc. fresh dog bile containing 68 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 2

*Dog bile feeding and bile secretion*

Dog 16–10. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | Weight | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 18 | | | | | 58 | | | | | 35.2 | 32.0 | Hemoglobin 99 per cent. |
| March 20 | 20 | 23 | 24 | | 67 | 12.2 | 11.4 | 12.4 | | 36.0 | 31.8 | Stools contain no stercobilin |
| March 21 | 22 | 55* | 53 | 27 | 135 | 11.4 | 10.0* | 6.0 | 6.7 | 22.7 | 31.8 | |
| March 22 | 26 | 27 | 21 | | 74 | 12.6 | 11.5 | 13.0 | | 37.1 | 32.0 | |
| April 12 | 18 | 24 | 28 | | 70 | 10.2 | 11.9 | 11.3 | | 33.4 | 31.0 | Stools contain no stercobilin |
| April 13 | 16 | 31† | 25 | 28 | 84 | 12.2 | 9.8† | 2.8 | 1.9 | 14.5 | 31.0 | |
| April 14 | 33 | 26 | 27 | | 86 | 10.4 | 9.4 | 9.1 | | 28.9 | 30.5 | |
| April 15 | | | | | 54 | | | | | 33.6 | 30.8 | Hemoglobin 104 per cent |

* 350 cc. fresh dog bile containing 130 mgm. bile pigment given through stomach tube at the beginning of the third hour.

† 200 cc. fresh dog bile containing 68 mgm. bile pigments given through stomach tube at the beginning of the third hour.

TABLE 3

*Dog bile feeding and bile secretion*

Dog 16–41. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | Weight | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 15 | 48 | 36 | 27 | | 111 | 10.5 | 11.2 | 11.5 | | 33.2 | 37.8 | |
| March 16 | 44 | 84* | 70 | 53 | 207 | 16.3 | 13.0* | 17.2 | 4.8 | 35.0 | 38.3 | |
| March 17 | 46 | 38 | 46 | | 130 | 13.1 | 12.1 | 12.7 | | 37.9 | 38.5 | |
| March 18 | | | | | 46 | | | | | 32.2 | 38.0 | |
| March 20 | 38 | 32 | 35 | | 105 | 12.0 | 10.8 | 10.2 | | 33.0 | 37.0 | Hemoglobin 94 per cent (Sahli) |
| March 3 | 30 | 36 | 33 | | 99 | 10.0 | 11.2 | 9.7 | | 30.9 | 37.3 | |
| March 4 | | | | | 90 | | | | | 30.6 | 37.5 | |
| March 6 | 37 | 25 | 31 | | 93 | 10.5 | 9.0 | 11.8 | | 31.3 | 36.8 | Hemoglobin 79 per cent |
| March 7 | 28 | 37† | 37 | 30 | 104 | 11.3 | 11.3† | 7.3 | 4.6 | 23.2 | 37.0 | |
| March 8 | 18 | 30 | 27 | | 75 | 9.4 | 12.1 | 9.7 | | 31.2 | 37.3 | |

* 450 cc. fresh dog bile containing 140 mgm. bile pigment given through stomach tube at beginning of the third hour.

† 150 cc. fresh dog bile containing 70 mgm. bile pigments given through stomach tube at the beginning of the third hour.

TABLE 4

*Dog bile feeding and bile secretion*

Dog 16–6. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | 1-2 hrs. | 3-4 hrs. | 5-6 hrs. | 7-8 hrs. | Total 6 hrs. | | Bread, bone and milk diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| Nov. 1 | 16 | 18 | 20 | | 54 | 10.8 | 13.8 | 11.9 | | 36.5 | 31.5 | |
| Nov. 3 | 17 | 18 | 20 | | 55 | 13.4 | 11.3 | 15.0 | | 39.7 | 31.5 | Stools contain no stercobilin |
| Nov. 4 | 22 | 20 | 22 | | 64 | 12.9 | 13.2 | 13.1 | | 39.2 | 31.0 | |
| Nov. 5 | 18 | 32* | 21 | 17 | 70 | 12.6 | 10.1* | 10.9 | 10.7 | 31.7 | 30.8 | |
| Nov. 6 | | | | | 66 | | | | | 34.1 | 30.8 | |
| Dog 16–27. Bile fistula and splenectomy | | | | | | | | | | | | |
| Feb. 26 | | | | | 32 | | | | | 22.2 | 26.0 | Mixed diet |
| Feb. 28 | 10 | 20 | 23 | | 53 | 7.5 | 10.4 | 8.2 | | 26.1 | 25.0 | Hemoglobin 81 per cent |
| Feb. 29 | 19 | 47† | 34 | 22 | 103 | 10.4 | 10.6† | 6.7 | 7.6 | 24.9 | 25.8 | |
| March 1 | 14 | 19 | 22 | | 55 | 8.8 | 8.9 | 9.4 | | 27.1 | 25.5 | |

* 45 cc. fresh dog's bile introduced into empty stomach through stomach tube at beginning of the third hour.

† 200 cc. fresh dog bile containing 140 mgm. bile pigment given through stomach tube at beginning of the third hour.

TABLE 5

*Dog bile feeding and bile secretion*

Dog 16–104. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| Feb. 25 | 20 | 15 | 17 | | 52 | 12.7 | 8.1 | 11.4 | | 32.2 | 33.0 | |
| Feb. 26 | | | | | 46 | | | | | 28.0 | 32.5 | |
| Feb. 28 | 14 | 17 | 18 | | 49 | 8.5 | 13.0 | 13.4 | | 34.9 | 32.8 | Hemoglobin 96 per cent |
| Feb. 29 | 16 | 44* | 28 | 23 | 95 | 10.0 | 13.8* | 7.0 | 8.8 | 29.6 | 32.5 | |
| March 1 | 25 | 19 | 19 | | 63 | 10.2 | 8.9 | 8.9 | | 28.0 | 33.0 | |

Dog 16–10. Bile fistula and splenectomy

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feb. 21 | 13 | 15 | 17 | | 45 | 10.6 | 11.0 | 11.8 | | 33.4 | 30.3 | Hemoglobin 94 per cent |
| Feb. 23 | 19 | 18 | 27 | | 64 | 10.4 | 8.1 | 11.6 | | 30.1 | 30.5 | |
| Feb. 24 | 12 | 40† | 40 | 34 | 114 | 9.6 | 11.3† | 10.5 | 6.2 | 28.0 | 30.3 | |
| Feb. 25 | 22 | 27 | 21 | | 70 | 9.4 | 12.8 | 11.5 | | 33.7 | 30.8 | |
| Feb. 26 | | | | | 42 | | | | | 30.4 | 31.0 | Stools contain no stercobilin |

* 100 cc. fresh dog bile containing 70 mgm. bile pigment given through stomach tube at beginning of third hour.

† 360 cc. fresh dog bile containing 198 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 6

*Dog bile feeding nine hours before collection*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | Bile pigments in milligrams | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | *lbs.* | |
| April 5 | 22 | 29 | 21 | 72 | 10.9 | 9.2 | 9.4 | 29.5 | 32.5 | |
| April 6* | 19 | 27 | 22 | 68 | 8.9 | 13.4 | 8.9 | 31.2 | 32.0 | |
| April 7 | 25 | 21 | 21 | 67 | 12.4 | 9.4 | 11.3 | 33.1 | 32.3 | Stools contain stercobilin |
| April 8 | | | | 54 | | | | 31.6 | 32.0 | |
| April 10 | 18 | 24 | 22 | 64 | 8.1 | 11.5 | 9.9 | 29.5 | 31.5 | Hemoglobin 116 per cent |
| Dog 16–27. Bile fistula and splenectomy | | | | | | | | | | |
| April 5 | 18 | 19 | 27 | 64 | 6.8 | 8.6 | 12.1 | 27.5 | 26.5 | |
| April 6* | 32 | 34 | 35 | 101 | 11.6 | 6.2 | 8.6 | 26.4 | 26.5 | |
| April 7 | 17 | 23 | 29 | 69 | 7.7 | 8.2 | 8.6 | 24.5 | 26.3 | |
| April 8 | | | | 50 | | | | 24.2 | 26.3 | |
| April 10 | 18 | 22 | 27 | 67 | 8.4 | 8.9 | 8.5 | 25.8 | 25.5 | Hemoglobin 94 per cent |

* 12.30 a.m. 300 cc. fresh dog bile containing 135 mgm. bile pigments given through stomach tube. Dogs set up at 9.30 a.m. Bile output nine hours after feeding dog bile.

TABLE 7

*Pig bile feeding and bile secretion*

Dog 16–27. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| | | | | | | | | | | | *lbs.* | |
| *1916* | | | | | | | | | | | | |
| March 22 | 18 | 17 | 24 | | 59 | 6.0 | 5.7 | 6.4 | | 18.1 | 26.3 | |
| March 23 | 29 | 24* | 13 | 13 | 50 | 8.6 | 3.8* | 1.2 | 0.9 | 5.9 | 26.8 | |
| March 24 | 35 | 40 | 23 | | 98 | 6.3 | 7.1 | 5.2 | | 18.6 | 26.5 | |
| March 25 | | | | | 40 | | | | | 25.4 | 25.8 | Stools contain no stercobilin |
| April 12 | 21 | 27 | 26 | | 74 | 6.2 | 7.9 | 6.4 | | 20.5 | 26.3 | |
| April 13 | 8 | 27† | 27 | 27 | 81 | 6.4 | 9.7† | 3.0 | 1.8 | 14.5 | 26.0 | |
| April 14 | 27 | 27 | 27 | | 81 | 7.9 | 6.0 | 6.7 | | 20.6 | 26.0 | |
| April 15 | | | | | 66 | | | | | 20.8 | 26.3 | Hemoglobin 95 per cent |

* 150 cc. fresh pig bile containing 65 mgm. bile pigment fed through stomach tube at the beginning of the third hour.

† 200 cc. fresh pig bile containing 68 mgm. bile pigments fed through stomach tube at the beginning of the third hour.

TABLE 8

*Pig bile feeding and bile secretion*

Dog 16–41. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 22 | 46 | 33 | 40 | | 119 | 10.4 | 9.7 | 9.0 | | 29.1 | 38.0 | |
| March 23 | 47 | 44* | 47 | 50 | 141 | 12.4 | 8.0* | 5.4 | 3.4 | 16.8 | 38.0 | |
| March 24 | 35 | 55 | 25 | | 115 | 11.0 | 14.1 | 7.3 | | 32.4 | 37.3 | |
| March 25 | | | | | 94 | | | | | 33.2 | 36.8 | |
| March 27 | 36 | 30 | 28 | | 94 | 14.4 | 13.6 | 10.6 | | 38.6 | 37.0 | Hemoglobin 93 per cent |
| Dog 16–60. Simple bile fistula | | | | | | | | | | | | |
| April 12 | 24 | 21 | 23 | | 68 | 6.4 | 4.7 | 6.2 | | 17.3 | 38.5 | |
| April 13 | 28 | 30† | 40 | 34 | 104 | 7.6 | 4.0† | 2.8 | 1.9 | 8.7 | 38.8 | |
| April 14 | 16 | 28 | 23 | | 67 | 5.4 | 10.6 | 3.1 | | 19.1 | 37.5 | |
| April 15 | | | | | 36 | | | | | 15 4 | 38.5 | Hemoglobin 98 per cent |
| April 17 | 20 | 19 | 21 | | 60 | 9.0 | 7.2 | 5.2 | | 21.4 | 38.3 | |

* 150 cc. fresh pig bile containing 65 mgm. bile pigment fed through stomach tube at beginning of the third hour.

† 200 cc. fresh pig bile containing 68 mgm. bile pigment fed through stomach tube at the beginning of the third hour.

TABLE 9

*Sheep bile feeding and bile secretion*

Dog 16–10. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 22 | 26 | 27 | 21 | | 74 | 12.6 | 11.5 | 13.0 | | 37.1 | 32.0 | |
| March 23 | 26 | 57* | 54 | 54 | 165 | 13.4 | 11.3* | 5.4 | 3.6 | 20.3 | 32.3 | |
| March 24 | 29 | 36 | 21 | | 86 | 12.4 | 15.8 | 10.9 | | 39.1 | 32.0 | Stools contain stercobilin |
| March 25 | | | | | 56 | | | | | 37.6 | 31.5 | |
| March 27 | 17 | 17 | 28 | | 62 | 13.0 | 8.8 | 15.2 | | 37.0 | 31.0 | Hemoblogin 97 per cent. |
| Dog 15–22. Simple bile fistula | | | | | | | | | | | | |
| March 22 | 35 | 35 | 26 | | 96 | 10.4 | 9.2 | 9.7 | | 29.3 | 31.5 | |
| March 23 | 22 | 58† | 40 | 14 | 112 | 10.4 | 10.2† | 3.6 | 7.6 | 21.4 | 31.5 | |
| March 24 | 35 | 35 | 21 | | 91 | 12.0 | 11.0 | 6.2 | | 29.2 | 31.5 | Stools contain stercobilin |
| March 25 | | | | | 94 | | | | | 28.8 | 32.0 | Hemoglobin 119 per cent. |
| March 27 | 22 | 26 | 25 | | 73 | 10.4 | 11.6 | 8.5 | | 30.5 | 31.5 | |

* 150 cc. fresh sheep bile containing 29 mgm. bile pigments given through stomach tube at beginning of the third hour.

† 150 cc. fresh sheep bile containing 29 mgm. bile pigments given through stomach tube at beginning of the third hour.

TABLE 10

*Ox bile feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | Mixed diet |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 10 | 24 | 25 | 25 | | 74 | 9.8 | 9.0 | 8.5 | | 27.3 | 31.3 | |
| March 11 | | | | | 64 | | | | | 26.4 | 31.5 | |
| March 13 | 20 | 25 | 19 | | 64 | 9.9 | 10.8 | 7.6 | | 28.3 | 31.3 | Hemoglobin 114 per cent |
| March 14 | 34 | 60* | 63 | 41 | 164 | 12.2 | 13.6* | 5.6 | 7.3 | 26.5 | 31.5 | |
| March 15 | 35 | 27 | 30 | | 92 | 13.3 | 8.2 | 10.7 | | 32.2 | 31.5 | |
| Dog 16–41. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 8 | 18 | 30 | 27 | | 75 | 9.4 | 12.1 | 9.7 | | 31.2 | 37.3 | |
| March 9 | 35 | 75† | 76 | 77 | 228 | 14.1 | 13.5† | 7.2 | 5.1 | 25.8 | 37.3 | |
| March 10 | 13 | 15 | 30 | | 58 | 10.2 | 7.7 | 12.8 | | 30.7 | 37.0 | |
| March 11 | | | | | 58 | | | | | 32.8 | 37.3 | |
| March 13 | 17 | 27 | 31 | | 75 | 9.2 | 10.3 | 9.8 | | 29.3 | 37.0 | Hemoglobin 81 per cent |

* 200 cc. fresh ox bile containing 51 mgm. bile pigments given through stomach tube at the beginning of the third hour.

† 250 cc. fresh ox bile containing 73 mgm. bile pigment given through stomach tube at end of the third hour.

Tables 7 and 8 show a pretty uniform reaction as regards the decrease in bile pigment secretion. The decrease is often over 50 per cent and the reaction very prompt, appearing usually in the first two hours. The fall during the two hour periods is remarkable, and indicates some very strong inhibiting substance which checks the outflow of bile pigments, while the volume of bile is unchanged or increased. Evidently pig's bile is different in some way from dog's bile as regards its physiological action on dogs.

Tables 9 and 10 show that sheep and ox bile have a tendency to depress the curve of bile pigment secretion but not comparable to the strong effect produced by feeding pig bile. Ox bile has the strongest cholagogue effect of any bile tested.

## DISCUSSION

The experiments tabulated above are pretty uniform, and in regard to certain points are capable of only a single interpretation. These experiments practically rule out any "bile circulation," meaning the absorption of bile pigment as such from the intestine. The experiments give no support to the theory of Addis that the pyrrol nucleus is absorbed from the intestine as urobilinogen, and carried to the liver as an important construction element for hemoglobin—a "bile circulation" of a pyrrol complex. In fact, these experiments give strong indirect evidence against this theory of Addis. The evidence is strong against the absorption of urobilinogen from the intestine, and all the known facts will fit a different theory, namely, that urobilin like bile pigments may at times be formed in one organ or another from some related pigment substance. Direct proof that urobilinogen can be absorbed from the intestine in considerable amounts is needed to render Addis' theory a useful hypothesis.

These experiments show the usual cholagogue action of fresh bile by mouth, but it is to be noted that even this well-known reaction is not absolutely constant. Ox bile in these experiments is the most active cholagogue, but all fresh bile will show considerable variation.

The inhibition of bile pigment output in spite of the cholagogue action is as constant as the direct cholagogue effect and equally pronounced. Fresh bile by mouth often doubles the bile flow and as frequently *decreases* by 50 per cent the outflow of bile pigments. Pig bile is most active in producing this inhibition of bile pigment secretion. The fall in output of pigments usually appears promptly, and the effect disappears within nine hours. We are not prepared to explain the

full significance of this constant reaction, but, to say the least, it strengthens our belief that bile pigments are formed in some other manner than through disintegration of red cells and hemoglobin.

As we find more substances (organic or inorganic) which influence promptly the secretion of bile pigments, may we not suspect that these pigments are manufactured in considerable degree by some organ which reacts promptly to various stimuli? It requires the widest stretch of the imagination to picture these various simple substances as influencing the red cell katabolism—one group causing prompt and extensive red cell destruction, and the other group equally prompt inhibition. Is it not very probable that these substances react on an organ busy in the production of pigments, and cause it to produce more or less pigment of a certain type?

## SUMMARY

Feeding fresh bile to bile fistula dogs causes an almost constant cholagogue action. Bile of the dog, sheep and pig all have this effect, and ox bile seems to be the most active cholagogue.

Fresh bile feeding as a rule causes a *fall in the output* of *bile pigments* in spite of the cholagogue action. Pig bile as a rule is most active in this respect, and may depress the bile pigment curve to less than half normal. Ox and sheep bile may have the same effect. Dog bile often causes only a slight fall in bile pigment output, but may depress the curve to 50 per cent of normal.

These experiments exclude the possibility of bile pigment absorption from the intestine. There is no evidence to support the theory of "bile pigment circulation." These experiments give no support to Addis' theory of pigment conservation, urobilinogen absorption from the intestine and synthesis toward hemoglobin. It is suggested that urobilinogen may be formed in other body tissues rather than absorbed from the intestine.

Splenectomy does not influence any of the above reactions in bile fistula dogs.

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# BILE PIGMENT METABOLISM

## V. The Influence of Bile Constituents on Bile Pigment Secretion, Taurocholic, Glycocholic and Cholic Acids and Bile Fat

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The preceding paper makes it clear that bile feeding may give somewhat variable results under pretty uniform conditions. We were particularly interested in the fact that pig bile almost always caused a remarkable decrease in bile pigment output in dogs with biliary fistulae, but dog bile had a less pronounced inhibiting effect or even no effect on bile pigment secretion. It is well known that bile of herbivorous animals differs in its chemical make-up from the bile of carnivorous animals. It was suspected that some of these variations in bile secretion following bile feeding might be explained by differences in chemical constitution of the ingested bile.

Any chemical substances capable of influencing the bile pigment secretion seemed worthy of investigation. Some of the more conspicuous elements of whole bile were isolated and tested in the experiments outlined below. That these substances have distinctive physiological activities is obvious, and that some of these have a definite action upon bile pigment secretion is equally clear. It is admitted that these experiments touch on only a few of the well known elements of normal bile, and many other constituents should be investigated. We hope to report further on these points in the future.

The experiments given below confirm those of the preceding paper in showing how profoundly and promptly the bile pigment output can be influenced by ingested substances. Further than that these experiments show that taurocholic acid has more of a cholagogue action than glycocholic acid but much less, if any, action on bile pigment secretion. Glycocholic acid is a less active cholagogue, but causes pronounced

inhibition of bile pigment output. Bile fat (simple ether extract) is also capable of depressing the curve of bile pigment secretion, while influencing in no degree the total output of bile.

It is probable that cholesterol is an inert substance as regards any control of bile pigment secretion. Some experiments were performed in which cholesterol (1–2 grams) were added to the daily food but with negative results. These experiments are not conclusive, and the question may be left open at present with a strong probability that cholesterol is a negative factor in bile pigment secretion.

## EXPERIMENTAL DATA

The same bile fistula dogs are used in the experiments as in the experiments described in the two previous papers. Unless note is made to the contrary, the same conditions obtain. The urine never contained more than a trace of bile pigments and no urobilin.

The taurocholic and taurocholeic acids were prepared from dog bile. The whole bile mixed with an excess of animal charcoal was evaporated to dryness at 70° to 80°C. The residue was extracted several times with absolute alcohol, and the alcoholic filtrate decomposed with hydrochloric acid and precipitated with ether. The bile acids were purified by redissolving in alcohol and reprecipitating with ether.

The glycocholic acid was prepared from fresh pig's bile, evaporating it to dryness in the presence of an excess of animal charcoal at 70° to 80°C., extracting the dried residue with absolute alcohol and precipitating the filtrate with dilute hydrochloric acid and water. The glycocholic acid was purified by redissolving in alcohol and precipitating with water.

The cholic acid used was prepared from glycocholic acid by hydrolysis with hydrochloric acid. The cholic acid fraction was rubbed up with distilled water, and then cold alcohol, and finally precipitated in relatively pure form from hot alcohol.

The bile fat was obtained from dog bile. The whole bile mixed with an excess of animal charcoal was evaporated to dryness, and the residue extracted with ether.

TABLE 21

*Taurocholic acid feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 17 | 33 | 32 | 27 | | 92 | 12.0 | 10.8 | 9.1 | | 31.9 | 30.8 | |
| March 18 | | | | | 52 | | | | | 32.8 | 31.5 | |
| March 20 | 25 | 24 | 31 | | 80 | 8.9 | 9.6 | 10.4 | | 29.9 | 31.5 | Hemoglobin, 116 per cent |
| March 21 | 19 | 53* | 29 | 19 | 101 | 9.4 | 10.6* | 9.2 | 9.9 | 29.7 | 31.5 | 5 grams taurocholic acid |
| March 22 | 35 | 35 | 26 | | 96 | 10.4 | 9.2 | 9.7 | | 29.3 | 31.8 | |

Dog 16–27. Bile fistula and splenectomy

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| March 31 | 10 | 20 | 27 | | 57 | 7.2 | 8.6 | 8.5 | | 24.3 | 26.5 | |
| April 1 | | | | | 44 | | | | | 18.8 | 26.5 | |
| April 3 | 17 | 22 | 29 | | 68 | 5.7 | 6.4 | 5.8 | | 17.9 | 26.0 | Hemoglobin, 92 per cent |
| April 4 | 22 | 44* | 36 | 30 | 110 | 5.9 | 6.0* | 4.8 | 5.2 | 16.0 | 26.5 | 3 grams taurocholic acid. |
| April 5 | 18 | 19 | 27 | | 64 | 6.8 | 8.6 | 12.1 | | 27.5 | 26.5 | |

* Taurocholic and taurocholeic acids dissolved in 400 cc. water given through stomach tube at the beginning of the third hour.

TABLE 22

*Taurocholic acid feeding and bile secretion*

Dog 16–10. Bile fistula and splenectomy

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| March 31 | 19 | 23 | 25 | | 67 | 11.0 | 12.9 | 12.4 | | 36.3 | 32.3 | |
| April 1 | | | | | 48 | | | | | 37.8 | 32.3 | |
| April 3 | 19 | 20 | 28 | | 67 | 14.1 | 11.6 | 15.5 | | 41.5 | 31.8 | Hemoglobin, 102 per cent |
| April 4 | 20 | 43* | 31 | 24 | 98 | 13.6 | 19.8* | 11.1 | 11.0 | 41.9 | 31.5 | |
| April 5 | 21 | 25 | 26 | | 72 | 12.2 | 12.9 | 14.0 | | 39.1 | 32.0 | |

Dog 16–41. Bile fistula and splenectomy

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| March 31 | 21 | 24 | 28 | | 73 | 8.4 | 9.2 | 9.4 | | 27.0 | 37.3 | |
| April 1 | | | | | 46 | | | | | 31.6 | 37.3 | |
| April 3 | 41 | 22 | 28 | | 91 | 12.2 | 8.9 | 8.8 | | 29.9 | 36.3 | Hemoglobin, 99 per cent |
| April 4 | 41 | 63* | 51 | 47 | 161 | 12.8 | 13.6* | 13.4 | 10.8 | 37.8 | 37.0 | |
| April 5 | 29 | 32 | 42 | | 103 | 9.2 | 10.4 | 11.8 | | 31.4 | 37.0 | |

* 3 grams taurocholic and taurocholeic acids dissolved in 600 cc. water given through stomach tube at the beginning of the third hour.

TABLE 23

*Glycocholic acid feeding and bile secretion*

Dog 16–60. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| April 6 | 24 | 7 | 45 | | 76 | 9.2 | 1.6 | 7.2 | | 18.0 | 38.3 | |
| April 7 | 22 | 16 | 29 | | 67 | 5.4 | 4.4 | 7.2 | | 17.0 | 38.8 | |
| April 8 | | | | | 66 | | | | | 22.4 | 38.3 | |
| April 10 | 20 | 14 | 29 | | 63 | 4.6 | 3.8 | 7.2 | | 15.6 | 37.3 | Hemoglobin, 101 per cent |
| April 11 | 23 | 30* | 27 | 35 | 92 | 5.7 | 2.7* | 1.8 | 2.4 | 6.9 | 38.5 | |
| April 12 | 24 | 21 | 23 | | 68 | 6.4 | 4.7 | 6.2 | | 17.3 | 38.5 | |
| Dog 16–27. Bile fistula and splenectomy | | | | | | | | | | | | |
| April 7 | 17 | 23 | 29 | | 69 | 7.7 | 8.2 | 8.6 | | 24.5 | 26.3 | |
| April 8 | | | | | 50 | | | | | 24.2 | 26.3 | |
| April 10 | 18 | 22 | 27 | | 67 | 8.4 | 8.9 | 8.5 | | 25.8 | 25.5 | Hemoglobin, 94 per cent |
| April 11 | 16 | 21* | 17 | 19 | 57 | 8.2 | 6.2* | 2.3 | 1.3 | 9.8 | 26.3 | |
| April 12 | 21 | 27 | 26 | | 74 | 6.2 | 7.9 | 6.4 | | 20.5 | 26.3 | |

* 10 grams glycocholic acid dissolved in 250 cc. 1 per cent sodium carbonate solution fed through stomach tube at the beginning of the third hour.

TABLE 24

*Glycocholic acid feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| April 7 | 25 | 21 | 21 | | 67 | 12.4 | 9.4 | 11.3 | | 33.1 | 32.3 | |
| April 8 | | | | | 54 | | | | | 31.6 | 32.0 | Stercobilin present |
| April 10 | 18 | 24 | 22 | | 64 | 8.1 | 11.5 | 9.9 | | 29.5 | 31.5 | Hemoglobin 116 per cent |
| April 11 | 26 | 34* | 23 | 26 | 83 | 10.0 | 10.0* | 4.1 | 3.0 | 17.1 | 31.5 | |
| April 12 | 15 | 23 | 27 | | 65 | 8.8 | 9 4 | 12.8 | | 31.0 | 31.0 | |
| Dog 16–41. Bile fistula and splenectomy | | | | | | | | | | | | |
| April 8 | | | | | 68 | | | | | 30.6 | 37.3 | |
| April 10 | 22 | 22 | 37 | | 81 | 10.4 | 10.4 | 13.7 | | 34.5 | 36.3 | Hemoglobin 110 per cent |
| April 11 | 27 | 29* | 53 | 37 | 119 | 9.8 | 11.0* | 2.5 | 3.1 | 16.6 | 37.0 | |
| April 12 | 21 | 28 | 27 | | 76 | 6.4 | 8.2 | 8.5 | | 23.1 | 37.3 | |

* 10 grams glycocholic acid dissolved in 250 cc. 1 per cent sodium carbonate solution fed through stomach tube at the beginning of the third hour.

TABLE 25

*Cholic acid feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | *lbs.* | |
| April 14 | 18 | 29 | 29 | | 76 | 11.3 | 13.1 | 12.4 | | 36.8 | 30.8 | |
| April 15 | | | | | 42 | | | | | 32.0 | 31.0 | |
| April 17 | 28 | 24 | 24 | | 76 | 9.4 | 9.2 | 11.5 | | 30.1 | 31.0 | Hemoglobin 118 per cent |
| April 18 | 15 | 22* | 27 | 27 | 76 | 6.1 | 10.4* | 9.4 | 8.9 | 28.7 | 31.0 | |
| April 19 | 29 | 29 | 26 | | 84 | 9.2 | 8.6 | 7.6 | | 25.4 | 32.0 | |
| Dog 16–41. Bile fistula and splenectomy | | | | | | | | | | | | |
| April 14 | 23 | 24 | 28 | | 75 | 10.9 | 11.0 | 10.0 | | 31.9 | 36.5 | |
| April 15 | | | | | 56 | | | | | 30.4 | 37.3 | Hemoglobin 106 per cent |
| April 17 | 20 | 23 | 28 | | 71 | 9.4 | 10 3 | 11.3 | | 31.0 | 36.0 | |
| April 18 | 19 | 29* | 15 | 21 | 65 | 3.4 | 6.5* | 3.0 | 4.6 | 14.1 | 37.0 | |
| April 19 | 22 | 29 | 32 | | 83 | 8.6 | 8.6 | 10.1 | | 27.3 | 37.5 | |

* 2 grams cholic acid emulsified in 200 cc. of a 1 per cent sodium carbonate solution given by stomach tube at beginning of third hour.

TABLE 26

*Bile fat feeding and bile secretion*

Dog 15–22. Simple bile fistula

| DATE | BILE | | | | | | | | | | WEIGHT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount in cubic centimeters | | | | | Bile pigments in milligrams | | | | | | Mixed diet |
| | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | 1–2 hrs. | 3–4 hrs. | 5–6 hrs. | 7–8 hrs. | Total 6 hrs. | | |
| *1916* | | | | | | | | | | | lbs. | |
| March 31 | 25 | 36 | 31 | | 92 | 9.0 | 12.0 | 9.1 | | 30.1 | 32.5 | |
| April 1 | | | | | 48 | | | | | 25.8 | 32.8 | |
| April 3 | 22 | 21 | 31 | | 74 | 10.4 | 11.5 | 10.4 | | 32.3 | 32.0 | Hemoglobin 116 per cent |
| April 4 | 25 | 23* | 23 | 22 | 68 | 8.5 | 5.2* | 5.7 | 5.6 | 16.5 | 32.3 | |
| April 5 | 22 | 29 | 21 | | 72 | 10.9 | 9.2 | 9.4 | | 29.5 | 32.5 | |
| Dog 16–27. Bile fistula and splenectomy | | | | | | | | | | | | |
| March 17 | 25 | 25 | 29 | | 79 | 8.5 | 7.3 | 7.2 | | 23.0 | 26.5 | |
| March 18 | | | | | 52 | | | | | 22.4 | 26.8 | |
| March 20 | 9 | 19 | 24 | | 52 | 8.8 | 8.5 | 8.6 | | 25.9 | 26.3 | Hemoglobin 85 per cent |
| March 21 | 20 | 17* | 17 | 20 | 54 | 6.4 | 4.6* | 3.8 | 3.9 | 12.3 | 26.5 | |
| March 22 | 18 | 17 | 24 | | 59 | 6.0 | 5.7 | 6.4 | | 18.1 | 26.3 | |

* 1.1 gram bile fat emulsified in 200 cc. water fed through stomach tube at the beginning of third hour.

## DISCUSSION

The above tabulated experiments make several points clear. Glycocholic acid has a moderate cholagogue action, but usually causes a great drop in bile pigment output in a bile fistula dog. Taurocholic acid has a strong cholagogue action, but little, if any, inhibiting effect on the bile pigment secretion. Bile fat (ether extract of whole bile) has no influence on bile flow, but causes inhibition of bile pigment secretion. Cholic acid has little effect on bile flow, but may decrease somewhat the bile pigment output.

With these facts in mind it may be possible to explain some of the obscure points noted in the preceding paper dealing with whole bile feeding. Pig bile is known to be very rich in glycocholic acid and very poor in taurocholic acid, so it is not surprising that pig bile given to bile fistula dogs usually causes a marked decrease in output of bile pigments. Dog bile is known to contain salts of taurocholic and taurocholeic acids but no glycocholic acid (at the most a trace). We understand now why dog bile does not cause as marked a decrease in bile pigment secretion as does pig bile. The ether extract of dog bile given to dogs with biliary fistulae usually causes a decrease in pigment output. This substance or group of substances may explain the tendency for dog bile feeding to cause a fall in bile pigment secretion. There may be substances in whole bile which are capable of increasing the outflow of bile pigments and their isolation and identification if present are much to be desired.

It may be objected that glycocholic acid is foreign to the dog's metabolism, and causes an unusual effect because of this fact. Such objections can not be raised against the other substances used, and the observations have some physiological interest.

## SUMMARY

Taurocholic acid given by mouth has a strong cholagogue action but little influence upon the secretion of bile pigments in bile fistula dogs.

Glycocholic acid by mouth has a moderate cholagogue action but a strong inhibiting effect upon bile pigment secretion.

Cholic acid has little if any cholagogue action, but may decrease somewhat the bile pigment secretion in the bile fistula dog.

Bile fat (ether extract) has no cholagogue action, but has a strong inhibiting effect upon the secretion of bile pigments.

The results obtained in bile fistula dogs by feeding whole bile may depend upon these factors (and others). The end result (inhibition or stimulation) is probably the true resultant of these many forces. It is possible to explain some of the divergent results met with after bile feeding by this study of some of the constituent parts of whole bile.

# OBSERVATIONS ON THE EFFECTS OF PARTIAL AND COMPLETE OCCLUSION OF BLOOD VESSELS ON THE GENERAL BLOOD PRESSURE IN MAN

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The effect on the general blood pressure of occluding and opening some of the larger arteries and veins in animals has been the subject of some study. So far as we are aware, however, observations of this kind have never been made in man. The present investigation was undertaken with a view to gathering information with regard to the effects on the general arterial pressure and heart rate of such procedures in man.

## DESCRIPTION OF APPARATUS AND METHOD OF PROCEDURE

The principal method consisted in applying different known pressures to the vessels of both thighs while following the arterial pressure in the brachial artery by the continuous oscillatory method (1). Both thighs, instead of only one, were used so as to obtain unmistakable reactions.

The apparatus consisted of an Erlanger sphygmomanometer; a foot plethysmograph with volume recorder; an air tank connected with a compressed air system, with a mercury manometer and with two blood pressure cuffs for the application of pressure to the thighs; and a time recorder.

The plethysmograph was made in the form of a boot large enough to include the foot and the leg up to about the middle of the calf with which an air-tight joint was made by means of a piece of band tubing. The volume changes were recorded by means of a calibrated volume recorder. The tank was charged with air at the pressure it was desired to throw into the cuffs on the legs. Then by simply turning a stopcock this pressure could be suddenly applied to or removed from the legs. Through the mercury manometer the moment of application

of pressure, the instant at which it was released and the time during which it was applied as well as the pressure itself were recorded. The effect upon the arterial blood pressure, upon the volume of the leg, the pressure exerted on the thighs and the time in seconds were recorded simultaneously.

The effect on the arterial pressure of applying the compression to and releasing it from the legs was followed by means of continuous blood pressure records made with the Erlanger sphygmomanometer according to a principle first employed by Erlanger (1). The subject of the experiment lay on his back upon a table; the right leg and foot were placed in the plethysmograph; a large blood pressure cuff was fastened around each thigh, as high up as possible; and the cuff of the Erlanger sphygmomanometer was placed on the left arm to record the changes in the amplitude of the oscillations obtained from the brachial artery. The tambour space of the sphygmomanometer was completely closed while recording the oscillations. Records to the number of 192 were made from two subjects at compression pressures ranging between 10 and 140 mm. Hg. The blood pressures of these subjects, both of whom were normal young men, were (E) average systolic pressure, 108 mm. Hg., average diastolic, 64; and (J) average systolic pressure 126 mm. Hg., average diastolic, 62.

## RESULTS

*Effect of compression on oscillation amplitude.* The effects noted varied with the level of the compressing pressure. Applying compression to the thigh at the lower pressures causes no change in the amplitude of the oscillations obtained from the brachial artery by the continuous method. At somewhat higher compression, an effect upon the oscillations first becomes apparent as an increase in amplitude. This increase in amplitude first occurs at a compression pressure of 70 mm. Hg. and at this pressure it is found in 12.5 per cent of the trials. It is observed in a larger proportion of the trials as the compressing pressure is raised until at 100 mm. Hg. it occurs in every one of the records. At 70 mm. the increase in amplitude is delayed, not following immediately upon the application of pressure, but appearing several seconds afterward (fig. 1). As the compression pressure is increased the delay in the appearance of the increased oscillations decreases until at 140 mm. all of the compressions cause an immediate and marked effect (fig. 2). These results are tabulated below (table 1).

At the lower compressing pressures the increase in amplitude is of short duration, usually disappearing in the course of a few pulse beats. At pressures above 110 mm., however, (fig. 2) it shows a greater tendency to persist and in a considerable number of the records the effect persisted without appreciable diminution until the pressure was re-

Fig. 1. Record to illustrate effect of a compression pressure of 70 mm. Hg. *1*, Volume of leg (right); *2*, compression pressure; *3*, sphygmomanometric record from left arm; *4*, time in seconds.

leased, the maximum duration being two minutes and twenty-two seconds.

*Effect of decompression on oscillation amplitude.* The effect of releasing the pressure is more marked than the effect of applying it; it becomes evident at lower compression pressures and the response is

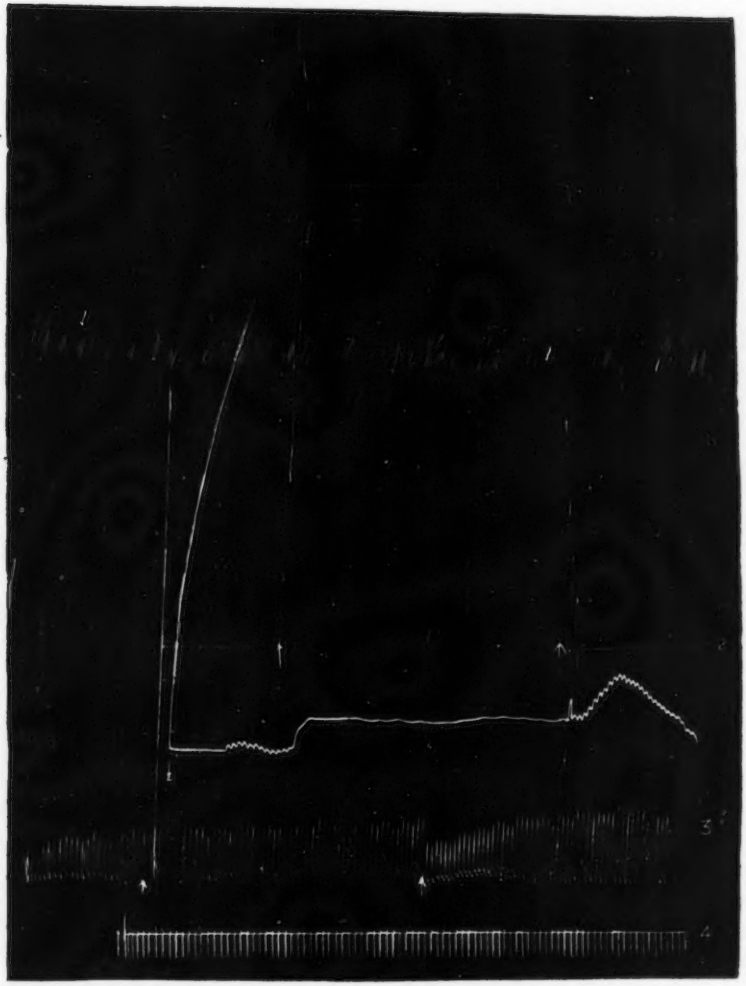

Fig. 2. Record illustrating effect of a high compression pressure. *1*, Volume of leg (right); *2*, compression pressure (140 mm. Hg.); *3*, sphygmomanometric record from left arm; *4*, time in seconds.

TABLE 1

| PRESSURE IN MM. HG. | PER CENT OF TRIALS SHOWING | | INCREASE IN VOLUME IN 15 SECONDS | TOTAL VOLUME INCREASE |
|---|---|---|---|---|
| | Increase in amplitude on compression | Decrease in amplitude on decompression | | |
| | | | cc. | cc. |
| 10 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 3.2 | 4 |
| 40 | 0 | Occasional, slight | 5.75 | 10.3 |
| 50 | 0 | Occasional, slight | 7.6 | 16.0 |
| 60 | 0 | Definite in 36 per cent, no delay. | 8.5 | 18.5 |
| 70 | Definite in 12.5 per cent, delayed. | Definite in 62.5 per cent no delay. | 9.7 | 24.0 |
| 75 | Definite in 33.3 per cent, not delayed. | Definite in 66.6 per cent | | |
| 80 | Definite in 28.5 per cent (Some delayed) | Definite in 85.7 per cent | 10.25 | 26.6+ |
| 90 | Definite in 57 per cent (Some delayed) | Definite in 85.7 per cent | 9.6 | 27.4+ |
| 100 | Definite in 75 per cent (Some abrupt) | Definite in 100 per cent | 9.7 | 29.0+ |
| 110 | Definite in 57 per cent (Some abrupt) | Definite in 100 per cent | 11.0 | 28.3+ |
| 120 | Definite in 100 per cent, abrupt | Definite in 100 per cent | 9.0 | 23.9 |
| 130 | Definite in 100 per cent, abrupt | Definite in 100 per cent | 3.8 | 5.5 |
| 140 | Definite in 100 per cent, abrupt | Definite in 100 per cent | 0 | 0 |

The data given in this table are based upon observations made on the subject (E). The observations made on (J) bear them out in every essential.

It must be remembered that the figures here given for total volume increase are only approximations and are much too low. More exact values might have been obtained with a different style of volume recorder.

The sign plus (+) where it appears in the table indicates that in a considerable number of cases the excursions of the recording lever were so wide as to cause it to leave the record. The values given are therefore very much too low.

The systolic pressure in the thigh was somewhat higher than that determined in the arm, averaging 114 mm. Hg.

immediate, despite the fact that the pressure is applied and released at approximately the same speed. The effect consists in a marked decrease in amplitude of the oscillations, and very often, more particularly at the higher pressures, a drop in the level of the base line of the record (figs. 1 and 2). This effect was noted with a pressure as low as 40 mm. Hg. and increased in frequency of occurrence with increasing pressure up to 100 mm. At and above this pressure it occurred in every case. The oscillations attain their lowest amplitude usually within one or two pulse beats and then return to their original size in the course of from 6 to 25 beats, usually about 12 beats. The effects of releasing the pressure are shown in table 1.

*Effect of the duration of compression.* For the purpose of determining the value of the time factor all the records made with compression pressures above 50 mm. Hg. were divided into groups, each group containing records having nearly the same compression pressure. Pressures below 55 mm. were not used because the effects at those pressures were very slight and thus difficult to measure, or absent altogether. There were nine groups in all. Group I contained records made with compressions of 55 to 63 mm. Hg.; group II, 68 to 75 mm. Hg.; group III, 75 to 80 mm. Hg.; group IV, 90 mm. Hg.; group V, 100 mm. Hg.; etc. up to group IX, 140 mm. Hg. An examination of all the records made with the object of determining the effect of the time during which compression was applied shows that the effect of releasing the pressure on the thighs varies directly with the length of time during which the pressure was applied in all the groups except I (55 to 63) and VIII (130). In group VIII, the time periods were not varied enough to form the basis of a fair comparison but as it appears in groups VII and IX it may safely be assumed that the time factor is present. Thus only group I is left in which no relation can be observed between the

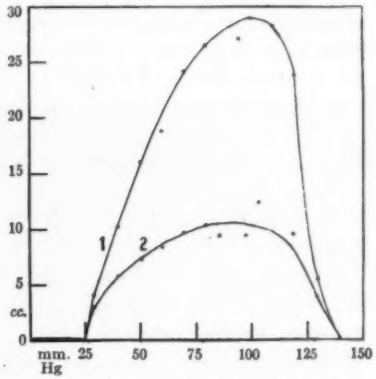


Fig. 9. Curves showing total volume increase (1) and increase in the first 15 seconds (2) at different compressing pressures. (Total volume increase for pressures of 80 to 100 mm. Hg. is too low. See footnote to table 1.)

length of time during which pressure was applied and the intensity of the effect of releasing it.

*Significance of the changes in amplitude.* That these changes in amplitude of the oscillations obtained from the brachial artery were indicative of changes in arterial blood pressure was definitely proven by a number of observations.

In the first place, to dispose of the possibility that the effects described might be due to any such cause as alterations in the position of the subject with resulting changes in the contact of the cuffs with extraneous objects, a series of experiments was performed in which the cuffs were so placed, and the body supported in such a manner that the blood pressure cuffs came into contact with nothing but the body. The records obtained in these experiments were in every respect similar to those obtained before.

Proof that compression and decompression cause changes in blood pressure was obtained by the use of the auscultatory method of determining the blood pressure. The pressure in the brachial artery was estimated by this method before and after compression, and again before and immediately after decompression. The compressions caused a rise in blood pressure of 4 to 8 mm. Hg., depending to some extent upon the pressure used. To detect the fall in blood pressure occurring as the result of decompression of the femoral vessels, the pressure in the brachial artery was determined about one minute after compression. Then, with the pressure in the sphygmomanometer about 2 mm. below systolic pressure, a pressure which just allowed the sounds to come through, the pressure on the thighs was suddenly released while listening to the arterial sounds in the brachial artery. The sounds disappeared. The pressure in the sphygmomanometer was then immediately allowed to fall, until the sounds returned. This occurred at a level which was from 4 to 14 mm. below the former systolic pressure. The extent of the fall in blood pressure depended upon the compression used, the most marked change occurring with the highest compression pressure.

In all of the experiments described above in which blood pressure records were made the pressure exerted on the brachial artery was a little below *systolic*. If the changes in amplitude observed are due to changes in blood pressure, we should expect, when the pressure in the sphygmomanometer is set at a level slightly lower than *diastolic*, compression and decompression to produce changes in the opposite direction to those observed in the records thus far described. For in that

case a rise of the systolic pressure, the pressure exerted on the brachial artery remaining constant, would increase the difference between the systolic pressure and the pressure exerted by the sphygmomanometric cuff, and the oscillations would diminish in amplitude (fig. 3). Conversely, a fall in the systolic blood pressure would be indicated by an increase in amplitude. Such a reversal in the effects would not occur if the changes in the amplitude of the oscillations were due to pulse pressure changes or to extraneous movements of the body.

The records made with this low pressure in the sphygmomanometer (60 to 75 mm. Hg.) showed very often a decrease in the amplitude of the oscillations upon applying the pressure to the legs, but never an increase. On releasing the compression, an increase was always observed, indicating that the effects were in reality due to changes in blood pressure (fig. 4).

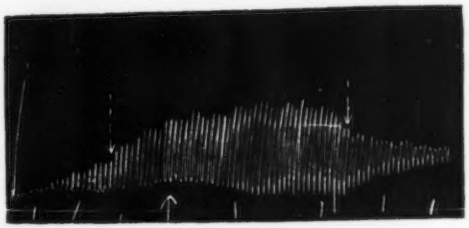

Fig. 3. Sphygmomanometric record to illustrate the principle of reversal. Arrows indicate pressure slightly below systolic, and slightly below diastolic, respectively.

Thus there can be no doubt but that the changes in amplitude of oscillations noted in the continuous blood pressure record actually indicate changes in blood pressure. Reading the changes of amplitude of oscillations in terms of blood pressure, it may therefore be concluded that compression of the thighs raises the arterial blood pressure, while decompression has the opposite effect. This effect is the more pronounced the greater the compression pressure used.

*Cause of the blood pressure changes.* The fact that the changes in oscillation amplitude were due to alterations of blood pressure having been established, the immediate cause of these blood pressure changes was next sought. Several factors had to be taken into consideration in this connection. In the first place, the blood pressure might be altered by vasomotor reflexes due to stimulation of the skin by the sudden application and removal of pressure through the cuffs. Again, the blocking of the venous flow which takes place particularly at the lower and medium pressures, with its storing up of blood in the legs and the subsequent infusion of this blood into the inferior vena cava

when the pressure is released might have an effect on the general arterial pressure. Alterations in blood pressure might also result from changes in the caliber of the arteries and hence in the resistance to the flow of blood through them brought about by compression and decompression.

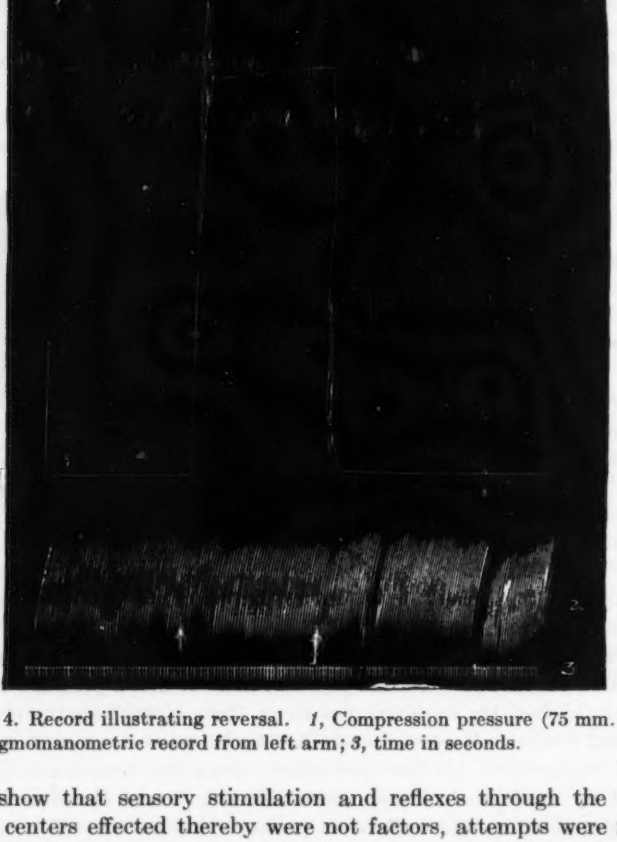

Fig. 4. Record illustrating reversal. *1*, Compression pressure (75 mm. Hg.); *2*, sphygmomanometric record from left arm; *3*, time in seconds.

To show that sensory stimulation and reflexes through the vasomotor centers effected thereby were not factors, attempts were made to supply the same sensory effects while protecting the femoral artery and vein from compression. This was accomplished by protecting the blood vessels of the thigh from compression by placing beneath the

cuff a piece of stiff wire netting bent so as to form a bridge over the vessels. That the flow through the thigh vessels was by this means preserved practically at the normal level was indicated by the fact that there was little or no increase in the volume of the legs when the compressing pressure was thrown into the thigh. Changes in amplitude of the oscillations in the sphygmomanometer record were slight or altogether absent, whereas in the records made with the same pressures but without the protection, the changes were considerable.

The effect of removal of blood from and addition of blood to the general circulation was investigated by means of the volume recorder which was connected with the plethysmograph. A compression of 10 to 20 mm. had no effect on the volume of the leg, excepting in one determination where a very slight accumulation of blood was noted. Usually accumulation of blood began to occur in the leg at 30 mm. Hg.[1]

The increase in volume of the leg consequent to compression is slow at low compression but the rate increases with the compressing pressure until it reaches approximately 80 mm. Hg. It then remains fairly constant until 110 mm. is reached. Above this pressure a rapid diminution in the rate of increase takes place. The total volume increase is greatest at 70 to 110 mm. pressure. Above 110 mm. the rate of accumulation of the blood decreased very rapidly with increasing compression, and at 140 mm. the flow of blood through the arteries was completely blocked. It is thus seen that the blocking of the arterial flow in the leg did not become complete until the compression pressure had risen 10 to 20 mm. above the systolic pressure as determined in the arm (see table 1 and fig. 9, p. 295). This is probably due in part at least, to the resistance of the tissues and the imperfect transmission of the compressing pressure through them.

The plethysmograph gave information with regard to volume changes in the foot and lower leg. Inasmuch as the volume of both legs from the position of the cuffs down is changing it therefore seems justifiable to assume that the total volume change is roughly four

[1] The increase in volume indicated by the plethysmograph under the influence of low compressing pressures is probably much too small, for the band tubing employed for the purpose of closing the plethysmograph exerts a certain amount of pressure on the leg. This will cause the veins in the part of the leg within the plethysmograph to fill with blood to the pressure exerted by the band tubing when a balance will be struck between inflow and outflow. This balance will be disturbed by compression of the thigh vessels practically only when this compression raises the venous pressure in the part of the leg without the plethysmograph above the venous pressure in the plethysmograph.

times the amount indicated by the recorder. The recorder had a maximum capacity of 40 cc. In many of the observations this capacity was exceeded. In such instances we are justified in assuming that the total accumulation of blood in the legs exceeded 160 cc.

The decrease in volume of the leg with decompression is very rapid. Never has more than twelve seconds been required for the leg to attain its initial volume, and usually by far the greatest part of the blood has left the leg within five or six pulse beats.

It may be assumed for reasons given above that decompression at low compressing pressures lets into the circulation a very much larger volume of blood than the figures in the table indicate. Since at very low compressing pressures (10 to 40 mm. Hg.) interference with arterial flow is certainly a negligible factor and since decompression at those pressures causes no or very slight alterations of arterial pressure it may be concluded that the addition to the general circulation of more than 40 cc. of blood in the course of two or three seconds is handled without affecting the general blood pressure.

Mention was made above of the fact that compression pressures of 40 to 50 mm. Hg. had given no increase of the oscillations on the sphygmomanometric record. Likewise several estimations made by the auscultatory method failed to show any fall in the pressure resulting from occlusion of the veins alone. It would therefore seem that in man the withdrawal of more than 64 cc. of blood as in this experiment does not affect the general blood pressure. It must be remarked, however, that these pressures are not those at which the maximum accumulation of blood in the legs takes place.

Whether the removal of the much larger volume of blood that occurs at somewhat higher compression pressures causes a fall of blood pressure we have not been able to determine because of the complication that results from the partial occlusion of the artery that then takes place.

*Effect of low compression pressures on the caliber of arteries.* If the conclusion is correct that the rise in general arterial pressure consequent upon the application of compression to the thighs is the result of an increase in arterial resistance, it follows that such low compression pressures as 50 or 60 mm. Hg. must diminish appreciably the caliber of the femoral arteries, otherwise the pressure changes determined by these slight compressions could not be explained on the basis of arterial resistance.

That low compressing pressures materially reduce the flow through

an artery was shown by a direct experiment in which a dog's carotid artery inserted in a circulation scheme was placed in a compression chamber by means of which any desired pressure could be exerted on the artery. Under a head of 66 mm. Hg. salt solution was allowed to flow through the artery as the compression pressure was lowered in steps from 68 mm. Hg. to zero. The results on the rate of flow through the artery are shown in table 2.

If we assume that the diastolic pressure in man is as in this experiment 66 mm. Hg., it is obvious that even so slight a compression as 15 mm. Hg. may result in a 22 per cent reduction in the diastolic flow, but only, of course, when the peripheral resistance is very low. It is also obvious that, owing to the diminution in the extensibility of the artery with increasing distention, the effects of compression will dimin-

TABLE 2

*Flow through the artery at different compression pressures*

Pressure head = 66 mm. Hg.

| TRIAL NO. | COMPRESSION IN MM. Hg. | FLOW IN CC. PER SECOND | CONDITION OF ARTERY |
|---|---|---|---|
| 1 | 68 | 0.0 | Artery closed |
| 2 | 63 | 0.38 | Artery open |
| 3 | 58 | 1.00 | Artery open |
| 4 | 54 | 1.5 | Artery open wider |
| 5 | 41 | 3.4 | Artery open wider |
| 6 | 28 | 4.96 | Artery open still wider |
| 7 | 15 | 6.92 | Artery open still wider |
| 8 | 6 | 8.08 | Artery almost full |
| 9 | 0 | 8.84 | Artery wide open |

ish with the increase in arterial pressure. As the result of this experiment we believe that the application of pressures less than diastolic to a limb may, under certain circumstances have a considerable effect upon the caliber of the artery of that limb and therefore upon the resistance to the flow of blood through the artery. This fact may have some bearing on the Hewlett method of determining the velocity of the blood flow in man.

*Attempt to dissociate arterial from venous effects.* The method described above having failed to show any marked changes in blood pressure resulting from occlusion of the veins alone, the attempt was made to differentiate arterial resistance effects from those due to the outflow of accumulated blood from the legs during decompression. A high

compressing pressure, 140 mm. Hg., under which no blood accumulated in the leg, was applied for fifteen seconds, then released and the effect noted. Now a low pressure, 70 mm. Hg., was applied for thirty seconds with its consequent damming back of blood in the legs, and was immediately followed by a high pressure, 140 mm. Hg., for fifteen seconds. This pressure was then released suddenly so as to produce the first effect plus an outflow of accumulated blood. The first procedure was then repeated. These experiments were repeated several times, varying the time intervals. No characteristic difference could be observed between the effect of a high compression alone and that of the same compression for the same length of time preceded by a

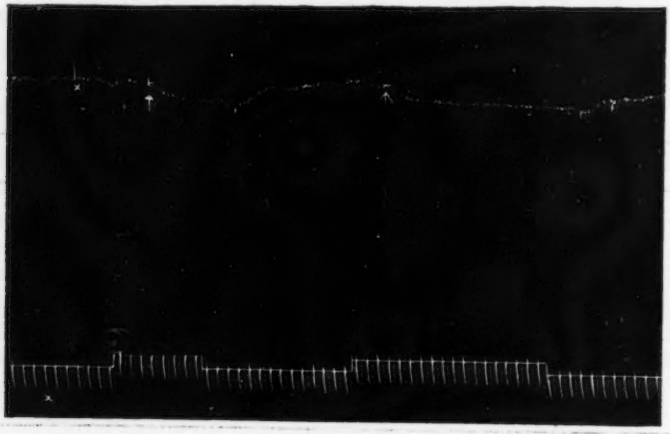

Fig. 5. Effect on arterial blood pressure of occlusion of inferior vena cava (cannula in carotid). Arrows indicate moment of occlusion.

low compression. These experiments seem to confirm the conclusion reached by the analysis of preceding results that the accumulation of blood in the legs and its sudden return to the circulation is not an important factor in the circulatory phenomena of compression and decompression of arteries and veins.

*Results with animals* An experiment was then performed on a rabbit for the purpose of determining the effect of these or at least similar procedures in animals. The abdomen was opened under ether anaesthesia, a ligature was passed around the inferior vena cava just above the junction of the common iliac veins, another around the

abdominal aorta, and a third around both vessels. The two ends of each ligature were drawn through a glass tube so that by pulling upon the projecting ends the vessel could be conveniently occluded. Upon compression of the vena cava the arterial pressure was observed to fall 6 mm. during the compression, somewhat more rapidly at first. Upon decompression the arterial pressure returned somewhat more rapidly to normal (fig. 5). Occlusion of the abdominal aorta caused the blood pressure to mount at first rapidly, then more slowly. The total rise was 10 mm. Hg. On decompression, the blood pressure fell rapidly to a point below normal and then slowly returned to normal (fig. 6). Compression of both vessels together caused an effect resembling that due to compression of the aorta alone. It differed from it slightly in that there was a small rapid rise in pressure followed by a fall, then a slow rise until decompression, which was followed by a fall in the blood pressure, a little slower than that due to aortic decompression, to a point below normal, then a slow return to normal (fig. 7). The total rise in this latter case amounted to 6 mm. Hg. The results obtained upon compression of the aorta alone and of the aorta and vena cava together are similar to those obtained upon aortic compression by Colson (2) in the dog and by Sollmann and Pilcher (3) in the dog and cat.

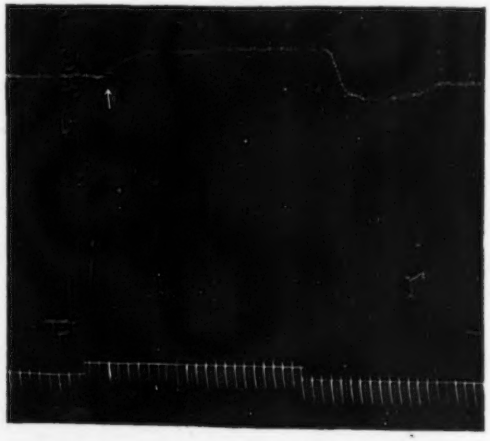

Fig. 6. Effect on arterial blood pressure of occlusion of abdominal aorta. Arrow indicates moment of occlusion.

Considered in the light of this experiment, the effects observed in man can be explained in the main as due to changes in arterial resistance. They may also have been affected, to some extent, by the blocking of the venous flow, the accumulation of blood in the legs, and its subsequent discharge into the circulation. Thus the delayed rise of pressure on compression with the lower pressures may as in animals

be due to venous obstruction, and the smaller fall in pressure due to decompression may be the result of the entrance of the accumulated blood into the system from the legs.

*Effect of compression and decompression upon the heart rate.* To determine the effect of compression and decompression upon the rate of the heart, 35 records were examined. The compression pressures used in these records ranged between 55 and 130 mm. Hg. Ten different periods consisting of five pulses each were examined in each record. These periods were so selected that the rate of the heart could be cal-

Fig. 7. Effect on arterial blood pressure of occlusion of both abdominal aorta and inferior vena cava. Arrow indicates time of occlusion.

culated immediately before the application of pressure, immediately afterward, midway between the application and release of the pressure, immediately before and after release of pressure, and at the end of the record. The heart rate was determined by counting the number of seconds in which the group of five pulse beats occurred.

Compression was followed by slowing of the heart rate in 55 per cent of the cases, no change in 25 per cent and some acceleration in 20 per cent. Decompression was followed by acceleration of the heart rate in 60 per cent of the cases, no change in 20 per cent, and slowing in 20 per cent.

Inasmuch as the rate must be calculated for very short periods, not over five seconds, it is obvious that not infrequently such changes in heart rate as may be caused by compression and decompression will be obscured by such concurrent changes in heart rate as occur in association with respiration, deglutition and similar processes. It is not surprising that the effect of compression and decompression on the heart rate is seemingly not always the same. The figures obtained therefore leave little room for doubting that compression retards and decompression accelerates the heart rate.

A few records were made with the kymograph moving at such a rate as to make possible the determination of the duration of each individual pulse. The results obtained in this way are essentially the same as those just mentioned. In addition, they show that the changes in the duration of the cardiac cycle follow immediately upon the changes of compression pressure.

The slowing of the heart beat upon compression can hardly be ascribed to anything but the rise of arterial pressure due to the increase in the arterial resistance. The acceleration observed following decompression can most satisfactorily be explained as due to the sudden fall of arterial pressure due to diminution in peripheral resistance, although the flow of the blood accumulated in the legs into the central venous system might conceivably play a part, especially as Bainbridge (4) has shown that the injection of blood or salt solution into the circulation of dogs, raising the venous pressure, is associated with acceleration of the heart, due to the increased venous pressure.

*Observations on a case of femoral arterio-venous aneurysm.* The opportunity presented itself of making some observations on a case of arterio-venous aneurysm in the Barnes Hospital which have a very direct bearing on the questions this paper deals with. A communication existed between the right femoral artery and vein. The effect of closing and opening the communication on the arterial pressure was determined. This was done by applying pressure over the site of the communication with the fingers while a continuous blood pressure record was being made with the Erlanger sphygmomanometer. The effect of closure came on within one or two heart beats and consisted in a tremendous rise of blood pressure and a slowing of the pulse rate (fig. 8). The blood pressure as determined by the auscultatory method rose in one experiment from 128 to 180 mm. Hg. Opening the communication caused a fall in blood pressure and an acceleration of the heart and restored the original conditions.

It is evident that in closing the communication we are shutting off the easy path of the blood into the veins and hence back to the heart, thus relieving the heart and indeed the entire venous system of an abnormally high pressure. At the same time the peripheral resistance is greatly increased. Opening the communication, of course, has the opposite effects. It seems fair to conclude that the changes in blood pressure here observed must be due to the changes in arterial resistance, as there seems to be no reason for believing that reducing the venous pressure and, for a time at least, the volume of blood supplied to the heart would cause the arterial pressure to rise (5).

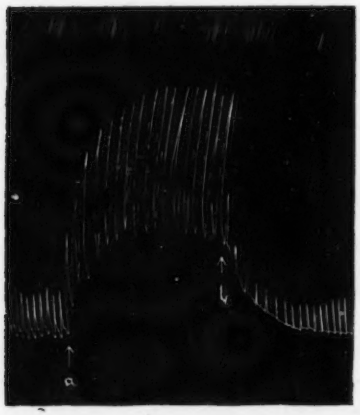

Fig. 8. Sphygmomanometric record from case of femoral arterio-venous aneurysm during closure and opening of the communication. Closed at *a* and opened at *b*.

The slowing of the heart rate observed on closing the arterio-venous opening was probably due to stimulation of the vagus center by the increased pressure, for at the operation after the patient had received atropine sulphate, there was no marked change in rate when the communication between femoral artery and vein was ligated. Some time after the operation, however, the heart rate slowed down to about 70 beats per minute, having been 95 per minute before it.

The phenomena observed in this case agree with those observed in the experiments in man and also in the rabbit. They may all be explained in the main, at least, on the basis of changes in arterial resistance.

*Compression of the arm.* In order to determine whether or not compression of the arm as in making blood pressure determinations affects the general blood pressure, the cuff of the sphygmomanometer was placed on the left arm and a continuous blood pressure tracing made while different pressures were applied to the right arm through another similar cuff. Pressures of 60 to 140 mm. Hg. were employed. No effects were observed. The results were such as to indicate that the changes in blood pressure resulting from the application of such a cuff to one arm, if they exist, are negligible.

SUMMARY

The effect of the occlusion of blood vessels upon the general blood pressure has been studied in man.

Circular compression of the thighs causes a rise in blood pressure, larger and more immediate at the higher than at the lower compressions. This is due to the increase in arterial resistance, modified somewhat at the lower compression pressures by the venous effect. The heart rate is somewhat slowed.

Decompression of the thighs causes a fall in blood pressure, more immediate and more profound than the rise due to compression. This effect is likewise more marked at the higher compression pressures and is modified slightly by the venous accumulation in the legs. The heart rate is somewhat accelerated.

The removal from the circulation of man of 64 cc. of blood does not change appreciably the arterial pressure.

A slight external pressure, even less than the diastolic pressure, upon an artery may under certain circumstances materially diminish the flow through it.

These results are in agreement with those obtained in the rabbit in so far as the arterial pressure is concerned, and in so far as it is possible to duplicate in man the conditions of an animal experiment.

The results of the experiments in man and in the rabbit in so far as the arterial blood pressure is concerned, are borne out by some observations on a case of femoral arterio-venous aneurysm.

The work was undertaken at the suggestion of Dr. Joseph Erlanger, and I wish to express my indebtedness to him for his advice and criticism during the preparation of this paper.

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# THE EFFECT OF ROTATION AND OF UNILATERAL REMOVAL OF THE OTIC LABYRINTH ON THE EQUILIBRIUM AND OCULAR REACTIONS IN KITTENS

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The ocular and other phenomena which present themselves after unilateral extirpation of the otic labyrinth in the adult cat and dog have been worked out in considerable detail by Wilson and Pike (1). These authors have found that immediately after recovery from the anesthetic, there is deviation of the eyes toward the side of the lesion, accompanied by nystagmus. This nystagmus, which is due in great part to the unbalanced activity of the intact labyrinth, consists of two phases: a slow deviation of the eyes to the side of the lesion, followed by a quick return to the initial position.

These ocular movements do not differ appreciably from those observed when the head is rotated in space and can be especially well studied in the post-rotatory period, in man as well as in certain other mammals. With the head in the vertex upward position there is a relatively slow deviation of the eyes in the direction of the preceding rotation with a quick return to the midline. In an extensive study of labyrinthine ocular movements in man, Bartels and Ziba (2) found that in the sleeping newborn and in prematurely born children (up to the seventh or eighth month) rotation of the head in space does not produce nystagmus. There is, however, a deviation of the eyes opposite to the direction of rotation during the rotatory period. On cessation of rotation the eyes return slowly to the position of rest.

As the study of the ocular movements following unilateral removal of the labyrinth has been confined to fully grown mammals, the observations of Bartels and Ziba at once suggest the study of these eye movements in very young animals under the same experimental conditions.

*Method.* The experiments were performed on kittens ranging in age from six days to seven weeks. The labyrinth was removed under

aseptic conditions according to the method described by Wilson and Pike (1). Great care was taken not to injure the cerebellum or cerebrum and cases showing such injury were eliminated from the series. In all cases the labyrinthine operation was preceded by rotation experiments. The animals were rotated about their ventro-dorsal axis by means of a turn table.

*Experiment I. Kitten six days old*

Palpebral membranes intact, but tore readily on forcible separation of the eyelids. Eyes midway between normal and embryonic position, i.e., with axes of vision markedly divergent. The animal crawls about unsteadily with limbs outstretched and with claws extended. When placed on its side, the animal returns to the standing position slowly and with apparent difficulty.

During the rotatory period, the head moves slightly opposite to the direction of rotation. In the post rotatory period, the head returns slowly to the mid line. No perceptible deviation of the eyes. No nystagmus.

Right otic labyrinth removed under ether. Slight torsion of the head with occiput to right. No apparent changes in equilibration. No appreciable change in the position of the eyes. No nystagmus. This animal was kept under observation for 36 hours. No changes were noted during this period. Death on the second day after the operation. Death in this case is attributed to exposure and inanition, as the mother avoided and refused to nurse the kitten following the operation. No signs of infection about operative wound.

*Experiment II. Kitten ten days old*

Eyes open for the last 24 hours. Walks about unsteadily with limbs outstretched, otherwise equilibration fair.

During the rotatory period the head moves opposite to the direction of rotation. During the post rotatory period the head moves in the direction of the preceding rotation for a few seconds, and then returns to the mid line with slow oscillatory movements. The eyes oscillate irregularly in a position of deviation in the direction of the preceding rotation. This effect is temporary, the eyes returning slowly to the position of rest. These oscillations do not present the alternating slow and rapid phases of well developed labyrinthine nystagmus.

(Another kitten 10 days old from the same litter, presented a slow post-rotatory nystagmus, about 6 cycles per minute, with the slow phase in the direction of the preceding rotation. The quick phase was not well marked, being only slightly more rapid than the slow phase. The head reactions did not differ in any respect.)

Left otic labyrinth removed. On recovery from the anesthetic there is marked torsion of the head (occiput to left). Practically no disturbances of equilibrium. The eyes deviate to the side of the lesion (fig. 1). No oscillation or nystagmus. At the end of 48 hours, position of head unchanged. Eyes still deviate to side of lesion, although the right pupil has shifted slightly toward the mid line. On the third day following the operation, this kitten was devoured by its mother.

*Experiment III. Kitten two weeks old*

Weight 220 grams. Slight unsteadiness of gait. In the post-rotatory period the head is carried in the direction of preceding rotation for a few seconds and then returns to the mid line. Irregular oscillations of the eyes, for a few seconds, in a position of slight deviation in direction of preceding rotation.

*June 1, 1916, 9.55 a.m.* Left labyrinth removed.

*10.20 a.m.* Marked torsion of head, occiput pointing to left. Animal shows tendency to fall to left side in walking. Eyes deviate to left and down (fig. 2), but no nystagmus.

*10.30 a.m.* At rare intervals slight oscillations of the eyes but no distinct nystagmus. Lateral nodding movements of head, slow to left, more rapid to right.

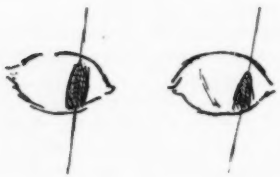

Fig. 1

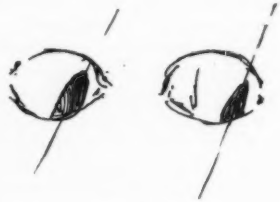

Fig. 2

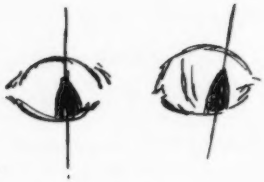

Fig. 3

*June 2.* Eye positions unchanged. No oscillations or nystagmus. Torsion of head as before. Equilibrium disturbances have disappeared to a great extent. When placed on its left side, the animal experiences difficulty in regaining the standing position. This is not the case when it is placed on its right side.

*June 4.* Equilibrium disturbances absent. Torsion of head unchanged. No ocular movements. Deviation greatly diminished, especially of the right eye (fig. 3).

*June 5.* Ocular deviation has entirely disappeared. Torsion of head as marked as before.

*June 7.* No change in behavior of animal since June 5.

*Experiment IV. Kitten three weeks old.*

Weight 275 grams. During rotation animal turns about its dorso-ventral axis away from the direction of rotation. The neck also bends in the same di-

rection. During the post rotatory period, there is turning of the body about its dorso-ventral axis and bending of the neck in the direction of preceding rotation. Slow post-rotatory nystagmus appears after a latent period. Slow phase in direction of preceding rotation and quick return in the opposite direction.

*August 28, 1916, 10 a.m.* Left labyrinth removed.

*2 p.m.* Animal shows tendency to turn about its dorso-ventral axis toward the side of the lesion. Occasionally falls in the same direction. Coarse vertical tremor of head. Marked torsion of head, occiput pointing toward the side of the lesion. Deviation of eyes in the direction of the lesion. At rare intervals slow oscillations of eyes or doubtful very weak nystagmus. When these movements appear the eyes oscillate about once every ten seconds.

*August 29.* Disturbances of equilibrium have entirely disappeared. Eye on the intact side shows slight decrease of deviation.

*August 31.* Deviation of eyes unchanged since last note. No ocular oscillations.

*September 9.* Observations since August 31 show progresssive diminution of deviation.

*September 11.* Deviation has entirely disappeared. Torsion of head unchanged.

*Experiment V. Kitten three weeks old*

Weight 266 grams. Animal shows distinct but slow post-rotatory nystagmus and the same general reactions as observed in kitten of Experiment IV.

*June 25, 1916, 3 p.m.* Right labyrinth removed. On recovery from the anesthetic the animal presents marked torsion of the head, occiput pointing in the direction of lesion. Marked deviation of eyes to side of lesion with very slow but distinct and irregular nystagmus.

*June 26.* Torsion of head unchanged. Deviation of eyes as before with slow nystagmus. The latter is often interrupted, the eyes remaining in the deviated position. Slight locomotor disturbances.

*June 28.* Locomotor disturbances have entirely disappeared. Torsion of head unchanged. Deviation of eyes not as marked as at last observation. Nystagmus has entirely disappeared. The eyes follow moving objects readily to the right but not to the left. There is absolute inability to turn the eyes past the mid line in the direction opposite to the operated side.

*June 29.* Deviation of eyes has entirely disappeared, but inability to turn the eyes past the mid line to left remains.

*Experiment VI. Kitten three weeks old*

Weight 283 grams. Rotation tests elicit the same symptoms described under Experiments IV and V.

*June 25, 1915.* Right labyrinth removed. Torsion of head to right. Nystagmus and deviation as in Experiment V. This animal however, showed practically no equilibrium disturbances. Lateral oscillations of the head occur at intervals of about five seconds. These head movements are slow toward the lesion but rapid and violent in the opposite direction.

*June 26.* Torsion of head unchanged. Deviation of eyes to side of lesion with occasional appearance of nystagmus at irregular intervals. The eyes

move slowly to the side of the lesion and return to the mid line distinctly faster. During the quiescent period the eyes remain in the position of deviation. Oscillations of head now occur at rare intervals.

From the 26th to the 29th of June this animal displayed exactly the same symptoms as the kitten of Experiment V during the corresponding dates.

*Experiment VII. Kitten four weeks old*

Usual responses to rotation. Post rotatory nystagmus of moderate speed. As in the kitten of three weeks there is a distinct latent period, unusually marked in this case, before the appearance of nystagmus. This latency will be referred to later.

*September 4, 1916, 10 a.m.* Left labyrinth removed.

*1 p.m.* Marked torsion of head and bending of neck to the side of the lesion. Occasional rapid oscillations of the head in the same direction, with slow return to the mid line. The animal moves about without difficulty, although there is a tendency to walk in a curve toward the left. Deviation of the eyes to the side of the lesion with slow but very distinct nystagmus (four to six oscillations per minute).

*September 5.* Very slight tendency to turn to the left in walking remains. Torsion of the head as before. Slow nystagmus present (about five oscillations per minute). The eyes move rather slowly to the mid line and after a few seconds return very slowly to the position of greatest deviation.

*September 6.* Nystagmus has entirely disappeared. Otherwise, no change since last note.

*September 9.* Deviation of the eyes has diminished gradually since September 8. The eyes are now in the mid line position, but there is apparent inability to turn the eyes to the extreme right.

*Experiment VIII. Kitten five weeks old*

Post rotatory motor disturbances and nystagmus well marked. The latter is rapid and occurs *immediately* after cessation of rotation.

*September 11, 1916, 5 p.m.* Left labyrinth removed.

*September 12, 8 a.m.* Marked torsion of the head to the side of the lesion. Animal turns to the left in walking. Oscillations of the head similar to those seen in the kitten of Experiment VII. Deviation of the eyes to the side of the lesion and nystagmus well marked. Rate of nystagmus: 15 per minute.

*5 p.m.* Nystagmus appears at irregular intervals. Rate unchanged during active periods. Deviation of the eyes unchanged.

*September 13.* Very slight deviation of the eyes remains. No nystagmus. Torsion of head unchanged.

*September 14.* Deviation of the eyes has entirely disappeared. There is, however, inability to turn the eyes past the mid line in the direction opposite to the side of the lesion. Torsion of the head unchanged.

*September 15.* No change since last note.

*Experiment IX. Kitten seven weeks old*

Reactions to rotation as seen in full grown cats. Rapid nystagmus and violent compensatory movements.

*June 1, 1916, 5 p.m.* Left labyrinth removed. Immediately after recovery from the anesthetic, torsion of the head to the side of the lesion appears. Deviation of the eyes to the side of the lesion with very rapid typical labyrinthine nystagmus. Tendency to turn to the left side in walking. Occasionally falls to the left. The head is carried violently to the right and then returns to the extreme left by a series of rapid oscillations.

*June 2.* The nystagmus has diminished in rate and is now interrupted by quiescent periods when only slight deviation remains. When the animal's attention is fixed, nystagmus is absent for a period of 40 to 120 seconds. Otherwise, the nystagmus movements occur in groups at intervals of about 10 seconds.

*June 3.* Nystagmus now occurs at infrequent intervals. Deviation of the eyes has almost disappeared. The animal seems unable to turn the eyes to the extreme right, i.e., in the direction opposite to the labyrinthine lesion. Oscillations of the head are now infrequent, and much less violent in character.

*June 4.* Deviation of the eyes, nystagmus and oscillations of the head have disappeared.

This animal was kept under observation for almost two months (until July 28). The inability to turn the eyes to the extreme right and the torsional effect on the head were the only persisting symptoms, as noted in several other animals. The torsion of the head in the direction of the lesion has been shown to result from loss of tonus in the cervical musculature on the side of the lesion (3). In view of this fact, the inability to turn the eyes to the canthi opposite to the side of the labyrinthine lesion, suggests the possibility of an asthenia in the recti muscles contralateral to the injury.

## SUMMARY

### *Rotation experiments.*

The reactions observed during the rotatory and post-rotatory periods increase, up to a certain limit, proportionally to the age of the animals. Kittens up to the second week after birth present only head and neck movements. After the third week the animal reacts bodily, turning on its ventro-dorsal axis. These general reactions increase progressively until the seventh week, at which time the behavior does not differ appreciably from that of the fully grown cat. As a whole, these reactions seem to progress *pari passu* with the development of equilibration.

Progressive changes are also seen in the ocular reactions. During the first two weeks after birth, the post-rotatory nystagmus is either absent, or when present, of a slow and irregular type. At the third week well defined labyrinthine nystagmus first becomes evident, the slow and rapid phases being distinguished without difficulty. The rate of nystagmus, slow in the younger animals, increases progressively until the fifth to the seventh week, when it does not materially differ from the post-rotatory nystagmus of the fully grown animal.

In animals of three to four weeks, the post-rotatory nystagmus is preceded by a latent period. For a few seconds after cessation of rotation the eyes, fixed in the median position, present a fine and very rapid horizontal tremor. The onset of nystagmus then occurs suddenly, the transition being extremely striking.

The following table shows the results obtained with a full grown cat, a five-week-old kitten, and one of four weeks, in which this latency was unusually pronounced. The animals were suspended by a string tied just below the fore limbs, and rotated at approximately the same speed. It will be noted that the latent period is absent in the older animals.

TABLE 1

| | DIRECTION OF ROTATION | PERIOD OF ROTATION | LATENCY | DURATION OF NYSTAGMUS |
|---|---|---|---|---|
| | | *seconds* | *seconds* | *seconds* |
| Kitten, Experiment VII, four weeks old | To left | 7 | 7 | 9 |
| | To right | 8 | 6 | 10 |
| | To left | 7 | 8 | 10 |
| | To right | 8 | 8 | 10 |
| Kitten, Experiment VIII, five weeks old | To right | 6 | 0 | 8 |
| | To left | 9 | 0 | 19 |
| | To right | 8 | 0 | 23 |
| | To left | 10 | 0 | 18 |
| Full grown cat | To right | 9 | 0 | 9 |
| | To left | 8 | 0 | 7 |
| | To right | 8 | 0 | 8 |
| | To left | 10 | 0 | 9 |

*The effects of unilateral removal of the otic labyrinth*

*Torsion of head.* With the exception of the six-day-old kitten in which the torsion of the head to the side of the lesion was slight, all the animals of the series presented torsion which did not differ from that seen in the full grown animals. This torsional effect, as shown by Wilson and Pike (1) is permanent. The kitten of Experiment IX, kept under observation for two months, showed no appreciable change in the posture of the head. As this torsion is due to impaired tonus of the cervical musculature on the side of the lesion (3), the absence of

marked torsion in the six-day-kitten may be attributed to late myelination of the labyrinthine tonus paths.

*Equilibrium.* The general disturbances of equilibrium following removal of the labyrinth, as in the rotation experiments, increase progressively with the age of the animals. As a whole the intensity of these symptoms is proportional to the developmental stage of the function of equilibration. Up to the second week after birth, these reactions are confined mainly to movements of the head. From the second week on equilibration disturbances of the body appear, which become progressively more violent until between the fifth and the seventh week, when these reactions are identical to those obtained in the fully grown cat. The equilibrium disturbances diminish in intensity after the first twenty-four hours and have entirely disappeared at the end of the second or third day.

*Ocular movements.* With the exception of the six-day kitten, in which no change in the position of the eyes was discernible, there is up to the end of the second week after birth deviation of the eyes toward the side of the lesion without distinct nystagmus. During the second week after birth, slight irregular oscillations of the eyes occur at intervals, but the fully developed two phase nystagmus does not appear until the third week. The nystagmus as seen in the three-week-old animals is of a slow type and interrupted. From the fourth week on it becomes regular. In the younger animals the oscillations occur at the rate of 4 to 6 per minute. This rate increases progressively, so that at the seventh week the rapidity of nystagmus approximates that seen in the fully grown animal.

In animals of three to five weeks, the nystagmus diminishes in intensity during the first twenty-four hours after the operation, and has entirely disappeared between the twenty-fourth and thirty-sixth hours. The deviation of the eyes on the other hand, does not begin to decrease appreciably until about the end of the forty-eighth hour, and persists for three to five days (in one case for ten days). In the seven-week-old kitten, as in fully grown cats, the deviation and the nystagmus disappear simultaneously.

If the ocular reactions following unilateral removal of the labryinth are compared with those observed in the rotation experiments, it will be seen that definite post-rotatory nystagmus occurs at an age (ten days to two weeks) when post-operative nystagmus does not appear. According to the views of Wilson and Pike (1) the nystagmus resulting from labyrinthine extirpation is due in great part to the unbalanced

activity of the intact labyrinth. This difference in reaction, therefore, seems to indicate that the stimulus derived from rotation is much more effective in producing compensatory eye movements.

Without wishing to emphasize the ontophylogenetic importance of some of the ocular reactions observed, the relative persistence of deviation and the absence of nystagmus in the younger animals seem to me suggestive, in view of the following facts:

1. The intensity of nystagmus, after unilateral removal of the labyrinth, varies as a rule with the position of the animal in the vertebrate phylum. While absent in the lower forms, its intensity is greatest in the higher mammals.

2. In the turtle, unilateral destruction of the labyrinth is followed by permanent deviation of the head to the side of the lesion (unpublished experiments by Professor Pike).

3. In prematurely born children rotation of the head in space is accompanied by deviation of the eyes *without nystagmus*. Observations of Bartels and Ziba (2).

Wilson and Pike (1) have shown that labyrinthine nystagmus is dependent on the integrity of two nervous paths, one associated with deviation, the other with the quick return. These authors have shown that the quick phase of nystagmus, after labyrinthine removal, can be eliminated by *complete* decerebration, including the optic thalamus. After this procedure deviation of the eyes alone remains. In another article the same authors (4) basing their conclusions on the work of Tozer and Sherrington (5) suggest that, "in labyrinthine nystagmus, the abnormal deviation of the eyes sets up sensory impulses in the afferent endings of the eye muscles themselves which bring about a return, either reflex or voluntary, of the eyes to the normal position. Nystagmus would then be a response to kinesthetic sensations." Accordingly, the absence or the undeveloped character of the quick phase, in the very young animals, may be explained by a relatively late myelination either of the kinesthetic paths related to the cerebrum and thalamus or of the efferent paths conveying impulses from the cerebrum and thalamus to the ocular muscles.

## CONCLUSIONS

1. In kittens, the general motor reactions which follow rotation and unilateral removal of the otic labyrinth are, up to a certain limit, proportional to the age of the animals. As a whole these reactions

seem to progress *pari passu* with the development of the function of equilibration.

2. The ocular reactions, likewise, show progressive changes. The compensatory eye movements following rotation of the head in space are at first of a slow and irregular type, but well defined labyrinthine nystagmus does not appear until about the third week after birth. The rate of post-rotatory nystagmus increases gradually with age.

3. In the younger animals post-rotatory nystagmus is preceded by a latent period.

4. Following unilateral removal of the otic labyrinth there is, up to the end of the second week after birth, deviation of the eyes to the side of the lesion without distinct nystagmus. At three weeks after birth nystagmus, of an intermittent type and of a slow rate, appears. The rate and regularity of the post-operative nystagmus, as in the rotation experiments, increases progressively with the age of animals.

5. In animals of three to five weeks the post-operative nystagmus disappears relatively early, whereas the deviation of the eyes to the side of the lesion persists for several days. In older animals the nystagmus and deviation disappear simultaneously.

6. The probable ontophylogenetic importance of these ocular reactions is suggested.

7. The ocular and other phenomena observed following rotation and unilateral removal of the otic labyrinth are explained by a relatively late myelination of the nervous paths concerned.

I wish to take this opportunity to thank Professor Pike for his kind assistance in the development of the operative technique used in these experiments.

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# STUDIES ON BIOLUMINESCENCE

## IV. The Chemistry of Light Production in a Japanese Ostracod Crustacean, *Cypridina hilgendorfii*, Müller

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CONTENTS



### INTRODUCTION

While a large and ever increasing number of organisms are known to produce light, in only a few of these is there sufficient light-giving substance to make chemical studies feasible. Luminous bacteria (1) are of practically no value, as also *Noctiluca* (2) and luminous squid; the fire-fly (3) and a pennatulid, *Cavernularia* (4) are somewhat better,

while the mollusc, *Pholas dactylus* (5), judging from Dubois' results, is better still. By far the most valuable of any of these organisms, if I may make comparisons without actual experience with *Pholas dactylus*, is the small crustacean, *Cypridina hilgendorfii*. For the size of animal, the light-giving substance is relatively enormous and its light-giving power incredibly great. Suffice it to say that one part of luminous gland substance in 1,600,000,000 parts of water will give visible light under proper conditions. In higher concentrations, the light is correspondingly stronger, plenty strong enough in fact, to be used for illuminating purposes could a sufficient quantity of the material be synthesized.

As Dubois (6) first demonstrated for *Pyrophorus* in 1885, so also in *Cypridina*, we may distinguish and separate two substances, in Dubois' terminology, luciferin and luciferase, which must be brought together before light will result. Contrary to my stand in previous papers of this series (7), I am now certain that Dubois' explanation of the action of luciferase and luciferin is quite incorrect, unless indeed light production be a different process in *Pholas dactylus* from that in *Cypridina* and the fire-fly. It is better perhaps to enter into a discussion of the rôle of luciferin and luciferase after the mechanism of light production and some of the properties of these two substances have been described.

The success of my investigations upon *Cypridina* and other luminous organisms was largely due to the interest and kindness of Prof. C. Ishikawa, of the Zoölogy Department, Agricultural College, Imperial University of Tokio, and to Prof. I. Ijima, of the Imperial University, who extended to me the hospitality of the Marine Biological Laboratory at Misaki. I am also indebted to Dean Kozai and Professor Aso of the Agricultural College for the use of many chemicals and apparatus. I extend my cordial thanks to all of these men.

## GENERAL CHARACTER OF LIGHT AND OF LUMINOUS GLAND

*Cypridina hilgendorfii* is a strongly negatively heliotropic ostracod best obtained at night from fish heads, on which the animals feed, lowered into the water. It may be caught the year round, but is most abundant during August and September in Sagami Bay, Japan. Another non-luminous species (*Cypridina x*) is often obtained from the fish heads together with *C. hilgendorfii*. It is positively heliotropic to lamp-light. *C. hilgendorfii* is so strongly negatively heliotropic that it is not readily caught on moonlight nights and avoids a shore with many electric lights.

The light-giving material of *Cypridina* is a secretion formed in a special gland of yellow spindle shaped cells, opening near the mouth.[1] In adult living *Cypridinas*, the gland cells form a cylinder about 0.64 mm. by 0.24 mm., having a volume of about 0.0003 cm.$^3$ The secretion is readily formed upon agitation of the animal and is easily visible in a room in the daytime. To the dark adapted eye, the color is decidedly bluish and the light very intense. To a partially light adapted eye, the color is blue. In this respect the light differs markedly from the yellowish white (to the dark adapted eye) light of luminous bacteria, of *Cavernularia*, or of the Japanese fire-fly, which is green to the partially dark adapted eye. There is no inhibition of the light secretion in daylight as has been described for other forms (8). When first caught, *Cypridinas* give off the secretion readily, sometimes upon mere contact with the surface film of water, but if they are kept in the laboratory the secretion is not given off so readily. Removal from sea water also inhibits the ejection of the secretion and it is necessary to squeeze the animal rather strongly before the secretion is given out.

Electrical stimulation also calls forth the production of an abundant light secretion.

## NATURE OF THE LIGHT SECRETION

The gland cells are almost completely filled with yellow material which can be observed to be composed of yellow globules 2 to 6 micra in diameter. These are extruded and dissolve to a colorless solution, absolutely free of visible granules, which gives the light. The globules are rather fluid in consistency as the outline can sometimes be seen to pass through amoeboid-like changes.

If we examine the natural secretion of *Cypridina* in sea water at night, the light appears perfectly homogeneous. No points of light appear such as might be due to granules, a condition wholly different from that in the juice of *Cavernularia* (4), which contains visible granules and at night the light from these under the microscope gives the appearance of a starry sky.

If *Cypridinas* be ground in a mortar and then examined at night, numerous very bright points of light appear and these are greatly increased by adding fresh water to the preparation. The light undoubt-

[1] For a description of the anatomy of the organ see Doflein, F., Sitzungsber. d. Ges. f. Morph. u. Physiol. in München., 1906, xxii, 133 and a paper by N. Yatsu in course of preparation.

edly comes from solution of the above described yellow globules and when they have dissolved no more points of light but a steady homogeneous glow is to be observed. Electrical stimulation by strong interrupted induced shocks does not intensify the light from an extract of *Cypridinas*, glowing faintly.

### PORCELAIN FILTRATION

Although no visible granules are found in the natural secretion of *Cypridina*, an ultramicroscopic investigation was not undertaken on account of lack of apparatus.

All of the light-producing substances (both photogenin and photophelein, p. 324) will easily pass a Pasteur-Chamberlain or a Berkefeld filter tube so that the particles present, if any, must be exceedingly small.

Complete proof of the truly soluble character of the light-producing substances is given by dialysis experiments recorded on page 329.

### LACK OF OXYGEN

Oxygen is necessary for light production as may be seen by placing the crushed animals in a hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of animal investigated likewise requires oxygen for phosphorescence.

### DESICCATION AND ETHER EXTRACTION

Like the fire-fly, the *Cypridinas* may be dried over $CaCl_2$ and will give a brilliant light if crushed and moistened with sea water. Dried crushed *Cypridinas* may be extracted with six changes of ether during the course of two days without impairing in the least their power to produce light when again moistened. The luminous substance is therefore of a non-lipoid, ether insoluble nature as might be expected from the fact that it is extruded from the animal as a clear water soluble non-fluorescent secretion. The luminous substance of the fire-fly and bacteria are also non-lipoid in nature (9).

## CHEMICAL TESTS ON THE SECRETION

Despite the fact that the light from the natural secretion of *Cypridina* is very bright a sample of the secretion, collected by shaking many *Cypridinas* in a small volume of sea water and filtering, responds to none of the common biochemical tests. It gives no precipitate with picric acid or on saturation with $(NH_4)_2SO_4$ or on boiling, even when made slightly acid. Fehling's reaction is negative as also the biuret and xanthroproteic for proteins, the Molish reaction for carbohydrates and the indophenol test (a-napthol and para-phenylen-diamine) + $H_2O_2$ for oxidases.

I do not mean to infer from this that the luminous substance is neither protein, fat, nor carbohydrate, but merely that the concentration giving a bright light is too small to respond to chemical tests (see p. 336).

## LUCIFERIN AND LUCIFERASE

The light from the natural secretion of *Cypridina* lasts for some time in sea water and then disappears and no amount of shaking will cause it to appear again. If we add to this natural secretion an extract of *Cypridina* heated to boiling, a brilliant light again results; or if we mix a water extract of *Cypridina* whose light has disappeared on standing with a similar extract whose light has been destroyed by boiling, light again results. In Dubois' phraseology we have mixed two substances, luciferin (in the boiled tube) and luciferase (in the tube allowed to stand) necessary for light production. According to Dubois, one of these, luciferase, is an oxidizing enzyme and is destroyed by heat; the other, luciferin, a substance not destroyed by heat is capable of oxidation with light production by means of luciferase. When the natural secretion of *Cypridina* is allowed to stand all the luciferin is oxidized and the luciferase is left; when a luminous extract of *Cypridina* is boiled the luciferase is destroyed before all the luciferin is oxidized.

At one time I believed Dubois' interpretation of this experiment to be correct, but results on *Cypridina* have led me to wholly different conclusions regarding the existence of luciferin and luciferase. Dubois' interpretation is indeed attractive. We know that the light production is an oxidation, that two substances are concerned, that these substances give light in very small concentration comparable with enzyme activity (see p. 336), that one of them can use up a large amount of

the other (see p. 337) and possesses certain properties (destruction by heat, phosphotungstic and tannic acid) characteristic of enzymes. Further, we actually know of oxidative reactions taking place with the production of light under the action of oxidizing enzymes from plants and animals (10).

It is quite possible that light production in *Pholas dactylus* is of this nature as it differs radically in very essential points from the mechanism in *Cypridina* and in the fire-fly. Thus, Dubois finds that *Pholas luciferin* (the substance not readily destroyed by heat and giving light with luciferase) can be oxidized with light production by many oxidizing agents—blood, $H_2O_2$, $KMnO_4$, $BaO_2$, $PbO_2$, etc.—and that it occurs only in the luminous organs of *Pholas dactylus* (5). I find that *Cypridina* luciferin (a substance not readily destroyed by heat and giving light with luciferase) will not give light with the above mentioned oxidizing agents and is found in many other non-luminous animals and in the non-luminous parts of *Cypridina hilgendorfii*.

Dubois finds that *Pholas* luciferase (a substance destroyed easily by heat and using up *Pholas* luciferin with light production) occurs in many other non-luminous animals (5) whereas I find a body with the same properties only in the luminous organs of *Cypridina*. This substance, which we may temporarily call *Cypridina* luciferase, in concentrated solutions, will give light (as before mentioned) with extracts of many non-luminous animals (see p. 326). It will also give light if mixed with many pure substances as chloroform, ether, benzol, thymol, saponin, oleic acid, atropin, NaCl and others. Since most of the above substances could not possibly be oxidized by the luciferase, I conclude that they cause in some way the giving out of light in what Dubois terms luciferase. On this view luciferase is the source of the light and the luciferin (in the boiled extract of *Cypridina*) is something which causes the luciferase to emit light.

The substances causing the emission of light from a concentrated solution of luciferase are similar to those producing cytolysis of cells and I have considered the possibility that the concentrated extract might contain fragments of the luminous gland cells which cytolyse with light production or possibly granules which dissolve with light production as described in many other forms and as especially prominent in the juice of *Cavernularia*. I am, however, convinced that there are in the extract of *Cypridinas* which will give light with unoxidizable substances no cell fragments present and no granules above those of submicroscopic colloidal dimensions, for the following reasons:

First, the light is always perfectly homogeneous and in marked contrast to the points of light of *Cavernularia* juice where visible granules and cell fragments do occur.

Second, *Cypridina* extract (luciferase) will give light with thymol, butyl alcohol, or NaCl crystals after filtration through a Chamberlain or a Berkefeld filter, which removes all cell fragments.

### PHOTOPHELEIN AND PHOTOGENIN

I conclude, therefore, that Dubois' luciferase, a thermolabile substance in luminous cells, is the light producing body. It gives light in contact with many substances not necessarily oxidizable and an especially bright light with a thermostabile (Dubois' luciferin) substance found in high concentration throughout the body of *Cypridina hilgendorfii* and in small concentration in the blood or juices of other non-luminous invertebrates. I therefore propose the new names photogenin (light producer from phos, light and gennao, to produce) for luciferase and photophelein (light assistor from phos, light and opheleo, to assist) for luciferin.

As we shall see later (p. 337) the photophelein can not be regarded as an enzyme because it is used up in the reaction and will not give light with an indefinite amount of photogenin. The photogenin corresponds much more to an enzyme but it is also slowly used up (p. 337). At present we can only speak of photophelein as a definite substance causing in some way the emission of light from photogenin.

Perhaps the following comparison of the important facts concerning light production in *Pholas* and *Cypridina* will make clearer Dubois' and my own views, and bring out the contradictory properties of the corresponding substances in the two animals.

| | PHOLAS | | CYPRIDINA | |
|---|---|---|---|---|
| | Luciferin | Luciferase | Photophelein | Photogenin |
| | Oxidizes with light production by luciferase | Enzyme accelerating oxidation of luciferine | Substance assisting in production of light by photogenin | Auto-oxidizable in presence of photophelein |
| Destruction by heat | − | + | − | + |
| Dialysable | − | + | − | + |
| Stability | + | − | − | + |
| Occurrence in non-luminous parts | − | + | + | − |
| Oxidized by $KMnO_4$, $H_2O_2$ etc. | + | − | − | − |

Whatever the exact interpretation of the facts may be, it is certain that two substances are concerned in light production and these may be separated because they are destroyed at different temperatures. We may now inquire into each of their properties separately and return to a discussion of the mechanism of light production, in considering the possible enzyme nature of photogenin.

## DISTRIBUTION OF PHOTOPHELEIN AND PHOTOGENIN IN ORGANISMS

Certain fundamental facts for the chemical theory of light production appear when we study the distribution of photophelein and photogenin —(I) in the non-luminous parts of *Cypridina;* (II) in non-luminous *Cypridinas* (*Cypridina x*); (III) in other non-luminous organisms; (IV) in luminous organisms.

By a careful quick scissors cut, the head end of *Cypridina* containing the luminous gland can be separated from the posterior half without any contamination of the latter with luminous secretion. If we now test the non-luminous half with dilute photophelein and photogenin, we find that it contains nothing which will give light with photophelein but something which will give a bright light with photogenin. We must try the experiment immediately because this substance disappears if the extract stands in presence of oxygen. In absence of oxygen or if the extract is boiled immediately (but not too long a time) the substance does not completely disappear even after one hour. There is, therefore, in the non-luminous parts, the substance photophelein which disappears even in the absence of photogenin (from luminous gland) unless the solution be boiled or oxygen excluded. Note the similarity to the disappearance of photophelein in presence of photogenin with this exception, that no light is produced. The experiment seems to indicate a third substance destroyed by heat, in non-luminous parts, which oxidizes (since oxygen is necessary) the photophelein.

In the extract of the non-luminous species, there is also a similar substance (photophelein) which will give light with photogenin. Unlike the photophelein from the non-luminous part of *Cypridina hilgendorfii*, it occurs in very small concentration, so that we must use concentrated[2] photogenin and concentrated extract of *Cypridina x*.

The photophelein from *Cypridina x* is also destroyed or disappears if the extract stands one and one-half hours in contact with oxygen

[2] By concentrated photogenin is meant an extract of one *Cypridina* in 0.5 to 1.0 cc. water.

but not in the absence of oxygen. Boiling makes the extract more stable.

In some other non-luminous forms widely different in relationship from *Cypridina*, there are substances which give light with concentrated photogenin and others which will not, whether the extract has been boiled or left unboiled. The extracts were boiled in order to destroy substances which in turn might quickly destroy photophelein. Among the light-giving extracts may be mentioned the following:

| ANIMAL | ORDER | CHARACTER OF LIGHT FROM JUICE | |
|---|---|---|---|
| | | Unboiled | Boiled |
| Sepia esculenta (blood) | Cephalopoda | Fair | Fair |
| Dolabella sp. (blood) | Gasteropoda | Faint | Fair |
| Laonome japonica (blood) | Annelida | Very faint | Very faint |
| Panulirus japonica (blood) | Decapodal | Very faint | Faint |
| Lepas anatifera (extract) | Cirripedia | Good | Bright |
| Tetraclita porosa (extract) | Cirripedia | Faint | Fair |
| Mitella mitella (extract) | Cirripedia | Faint | Fair |
| Oxyrhynchidae (moss crab) (extract) | Decapoda | Faint | Fair |
| Palaemon sp. (extract) | Decapoda | Faint | Faint |
| Chiton (extract) | Amphineura | Fair | Good |
| Harmithoe imbricata (extract) | Annelida | Fair | Fair |
| Cirratulus dasylophyus (extract) | Annelida | Very faint | Very faint |
| Marphysa iwamusi (extract) | Annelida | Faint | Faint |
| Onchidium sp. (extract) | Gasteropoda | Faint | Faint |
| Non-luminous parts fire-fly (extract) | Insecta | Negative | Fair |
| Flat worm (sp?) (extract) | Turbellaria | Faint | Faint |

Of these forms *Lepas* and *Chiton* gave the best light and of these two only *Lepas* gave light with dilute *Cypridina* photogenin. Too much stress must not be laid upon comparative results because much depends upon the concentration and it is not easy to obtain extracts of comparative concentration.

Unlike the photophelein from the luminous parts of *Cypridina* or from non-luminous *Cypridinas*, that of *Lepas* extract or of *Dolabella* blood is perfectly stabile and will give light with photogenin even after standing a period of 24 hours.

Many extracts were found to give no light with concentrated photogenin. These included the following, which were tried both boiled and unboiled. Again it is possible that with greater concentrations even these extracts would call forth a faint light.

| *Animal* | *Order* |
|---|---|
| Modiolus sp. ? | Lamellibranch |
| Acmaea sp. ? | Gasteropod |
| Sistrum sp. ? | Gasteropod |
| Sphaerechinus pulcherrinus | Echinoid |
| Wall crab (sp. ?) | Decapod |
| Ligia exotica | Isopod |
| Coccinella 7-punctata | Coleoptera |
| Anomala rufscuprea | Coleoptera |
| Glysiphana jocunda | Coleoptera |

The following fluids and dissolved protein substances also give no light when their solutions were mixed with photogenin.

| | |
|---|---|
| 50 per cent egg albumen | Witte's peptone |
| 50 per cent egg yolk | Neutral meat peptone |
| Na nucleate | Dried ox blood extract |
| Na nucleoproteinate | |

It is certain, then, that there is photophelein or something similar to it in the blood or extracts of many invertebrates but not necessarily in solutions of protein substances such as egg albumen, peptone, etc. In saliva there is something giving a very faint light and something in urine giving a fairly bright light with photogenin. Certain fluids tried were sufficiently acid to prevent the appearance of light and some fortuitous characteristic such as acidity may explain why extracts of some invertebrates give no light with photogenin.

It is hardly worth inquiring into the nature of the substances in each particular extract which may for convenience be collectively spoken of as photophelein since I have found a great many simple bodies, which, mixed with concentrated photogenin in powder or crystal form, give rise to a bright light. With more dilute solutions (one *Cypridina* to 10 cc.) no light appears. These substances include thymol, chloroform, hydrochinon, NaCl, butyl alcohol, saponin, oleic acid, ether, benzol, atropin, pilocarpin and ortol. Chloral hydrate, pyrocatechin, $MgSO_4$, $(NH_4)_2SO_4$, aesculin, dextrine and $K_4Fe(CN)_6$ gave a fainter light while saccharose gave none.

It is difficult to see in just what way all these diverse substances act. One is inclined to compare the production of light by chloroform or saponin to a process of cytolysis or to a stimulus reaction—like artificial parthenogenesis. The photophelein of *Cypridina* or other extracts would then be comparable to the cytolytic substance in the blood of worms which causes development of sea urchin eggs (11). The

chloroform or saponin might be supposed to cause the solution of granules or globules (as can be observed in many cells) and the production of light to be connected with this solution process. But I have already given evidence (p. 324) to show that there are no granules unless ultramicroscopic ones in the light secretion of *Cypridina*, so that we must look to another interpretation.

Of many animal extracts tried on both concentrated and dilute photophelein, none will give light. Neither have I been able to obtain light with any chemicals or the oxidizing substances; neutral $H_2O_2$, $BaO_2$, ox blood alone or with neutral $H_2O_2$ added, neutral potato juice alone or with neutral $H_2O_2$, $KMnO_4$, $K_2Cr_2O_7$, $FeCl_3$, or $K_4Fe(CN)_6$. As already pointed out (p. 323) this result is the direct opposite of the case in *Pholas dactylus* as described by Dubois.

There is, then, in the non-luminous parts of *Cypridina hilgendorfii* a large amount of photophelein but only a small amount even in a closely related non-luminous form. There appear to be only small amounts in other luminous forms not closely related to *Cypridina*. For instance, *Cypridina* photogenin will give a faint light with fire-fly photophelein but it is not nearly so bright as with *Cypridina* photophelein nor as bright as that given by non-luminous *Lepas* extract. Unfortunately, no closely related luminous crustacean was available so that the degree of specificity of photogenin and photophelein could not be determined. At least we may say that *Cypridina* photogenin and photophelein are specific to a certain extent, but not in the strict sense of the word.

The following tabulations give the luminous animals which have been tried with *Cypridina* in concentrated solution and the character of the light resulting. None gives light in more dilute solution.

| | |
|---|---|
| Cypridina photophelein × Fire-fly photogenin | faint |
| Fire-fly photophelein × Cypridina photogenin | faint |
| Fire-fly photophelein × Fire-fly photogenin | bright |
| Cypridina[3] photophelein × Cavernularia photogenin | fair |
| Cavernularia photophelein × Cypridina photogenin | negative |
| Cavernularia photophelein × Cavernularia photogenin | negative |
| Cypridina photophelein × Noctiluca[4] photogenin | faint |
| Noctiluca[4] photophelein × Cypridina photogenin | faint |
| Noctiluca[4] photophelein × Noctiluca[4] photogenin | negative |

[3] An extract prepared with sea water, as the dark juice of *Cavernularia* gives a brilliant light with fresh water.

[4] All the *Noctiluca* extracts were made with n/1000 $NH_4OH$ to neutralize the acid of the *Noctiluca* vacuoles.

Owing to lack of material, the luminous fish, *Monocentris japonica* could only be tested in more dilute solutions, and with negative results, viz.:

Cypridina photophelein × Monocentris photogenin.....negative
Monocentris photophelein × Cypridina photogenin.....negative
Monocentris photophelein × Monocentris photogenin.....negative

### DIALYSIS

Photophelein will dialyze through heavy parchment and collodion fairly readily, in the case of collodion sometimes appearing in the dialysate in the course of two hours.

Photogenin dialyzes with difficulty and with some collodion tubes not after a period of thirty-six hours. In others a very slight amount will pass in that time and in one experiment with heavy parchment paper a very slight dialysis occurred in twelve hours but usually there was none. The collodion tubes and the parchment paper did not leak in any of the experiments recorded.

### ADSORPTION

Both photophelein and photogenin are removed from solution by washed boneblack and washed freshly precipitated $Fe(OH)_3$. To serve as control the last washings from boneblack and $Fe(OH)_3$ were added to photogenin and photophelein and tested with photophelein and photogenin respectively to make sure that the adsorption was not apparent and due to destruction by foreign substances from the adsorbing media.

### TEMPERATURE

The exact temperature of destruction of photophelein and photogenin depends upon their concentration and the time of heating. *Cypridinas* dried over $CaCl_2$, ground, and the powder suspended in sea water give a beautiful light which disappears when heated to 56°, but returns on cooling. If heated to 65° and cooled, the light also returns, but does not return if heated to 70° and then cooled. A very concentrated mixture of photogenin and photophelein may be heated above 70° and whole *Cypridinas* heated to boiling will occasionally give a faint light when cooled. The light from the normal secretion of *Cypridina* disappears at 52°–54°, and returns on cooling, so that we may regard this as the inhibition temperature and something above 70°, depending on the concentration, as the destruction temperature.

Other organisms give the following inhibition or destruction temperatures:[5]

| | |
|---|---|
| Luminous bacteria (17) 38° | Recovery if cooled immediately |
| Hydroid, Sertularia 54° | No recovery on cooling |
| Noctiluca (2) 48° | No recovery on cooling |
| Cavernularia (4) 52° | No recovery on cooling |
| Luciola (3) 42° | No recovery on cooling |
| Pholas dactylus (5) 60° | Recovery? |

The time necessary to destroy photophelein at 100° depends also upon its concentration. In dilute solution (one *Cypridina* to 25 or 50 cc. water) boiling for one minute is sufficient but in concentrated solution (one *Cypridina* to 1 cc. water) five minutes boiling is necessary.

In *Pholas dactylus* (5) photophelein (luciferin) is destroyed above 70° whereas in the fire-fly (both *Photuris* and *Luciola*) it may be boiled for ten minutes without destruction.

The luminous material of *Cypridina* like that of most luminous organisms is unaffected by cold and will glow brilliantly at 0°C.

## STABILITY

*Cypridina* photogenin is much more stabile than photophelein. The time after preparation that one can obtain light from these substances depends largely upon their concentration as also upon the temperature. One sample of concentrated (one *Cypridina* to 2 cc.) photogenin gave light with fresh photophelein for seven days at 20° despite the fact that decomposition had taken place and the liquid smelt foul. Dilute photogenin (one *Cypridina* to 50 cc.) may lose its power in one day; very dilute photogenin (one *Cypridina* to 12,500 cc.) in less than two hours.

*Cypridina* photophelein also disappears from solution spontaneously, and the more dilute the solution the more quickly does it lose its power to phosphoresce. With one *Cypridina* to 25 cc. the power disappears in three to four hours, while with one *Cypridina* to 1 cc. it may last for five days at 26° to 28°C.

The decomposition occurs whether the solution be neutral, acid (n/8000 HCl) or alkaline (n/4000 NaOH), in pure water or sea water, in the light and also in amber bottles, and occurs even if the photophelein be thoroughly boiled to destroy all traces of photogenin which

[5] See also the table in Mangold's article, Die Production von Licht, in Winterstein's Vergleichende Physiologie, Vol. 3, part 2, 345.

might slowly use up the photophelein without light production (see p. 337).

The photophelein is apparently oxidized, as the destruction is not so rapid in absence of oxygen.

### PRESERVATIVES AND ANESTHETICS

The addition of preservatives (anesthetics) as chloroform, ether, benzol and thymol is to hasten the spontaneous destruction of the photophelein and to preserve the photogenin. Ether is especially destructive to the photophelein whereas photogenin will give light after saturation with ether for twenty-two days. The following tables (1 and 2) show the effect of saturation of solutions (one *Cypridina* to 25 cc.) of photophelein and photogenin with the four substances.

TABLE 1

*Effect of preservatives on photophelein*

| SATURATION WITH | TESTED WITH PHOTOGENIN AFTER | | | |
|---|---|---|---|---|
| | 5 minutes | 1 hour | 1 day | 2 days |
| Ether | Bright light | Negative | Negative | Negative |
| Benzol | Bright light | Very, very faint | Negative | Negative |
| Chloroform | Bright light | Very faint | Negative | Negative |
| Thymol | Bright light | Faint | Negative | Negative |
| Control | Bright light | Bright light | Faint[6] | Negative |

TABLE 2

*Effect of preservatives on photogenin*

| SATURATION WITH | TESTED WITH PHOTOPHELEIN AFTER | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | 2 days | 7 days | 22 days | 56 days | 130 days |
| Ether | Bright | Bright | Faint | Very faint | Negative | |
| Benzol | Bright | Bright | Bright | Negative | | |
| Chloroform | Bright | Bright | Bright | Bright | Bright | Negative |
| Thymol | Bright | Bright | Faint | Faint | Negative | |
| Control | Bright | Faint | Negative | | | |

The harmlessness of the above anesthetics for *Cypridina* photogenin is unusual as Dubois found a marked destructive action on *Pholas* photogenin (luciferase) and I have noted the same thing for the fire-fly.

[6] Often photophelein will not last for one day.

As we have just seen, the addition of certain anesthetics does not rapidly destroy photophelein or photogenin. We can saturate a phosphorescent mixture of the two with ether and the light will still last for some time. If we add butyl alcohol to saturation the light disappears and if the solution is now diluted with water or sea water, *the light reappears.* The same phenomenon is observed if the photogenin be filtered through a Chamberlain porcelain filter to remove all traces of cells or cell fragments. Care was taken to make sure that the return of light was not due to fluid adherent to the sides of the test tube and untouched by the butyl alcohol.

A similar phenomenon is observed with ethyl alcohol and acetone. If we add in small amounts absolute ethyl alcohol to a glowing mixture of photogenin and photophelein, the light becomes very dim when 16 per cent alcohol has been added and disappears with 20 per cent alcohol. If now the mixture be diluted the light returns. Acetone[7] behaves as alcohol. About 23 per cent is necessary for extinction of the light. Saturation with chloretone does not extinguish the light.

The effect of ethyl alcohol and acetone might be explained as the effect of precipitation, because of insolubility in the 20 per cent solution but we can not so explain the extinction of the light by butyl alcohol and subsequent recovery on dilution since butyl alcohol is only soluble to the extent of 8.3 parts in 100 parts of water. We are dealing with a highly interesting effect—one akin to anesthesia—a reversible inhibition, not of a cell or cell fragments but of a solution. Filtration through porous porcelain shows that no cell fragments can be present. I can not here enter more fully into a discussion of this interesting phenomenon, whose bearing on anesthesia is obvious, except to point out that if we can anesthetize a solution, we need not, as some recent theories have done, regard changes in the cell membrane to be necessarily the ultimate cause of anesthesia.

It may be pointed out in passing that the production of light gives us the opportunity of observing the effect of, let us say, an anesthetic or temperature, upon a process at any particular instant, rarely obtained in the study of enzyme action or even of cell action except where some movement is affected. For instance, we can heat pepsin to 60° and then cool it and see if it will digest protein. If we find that it will digest protein, that gives us no information as to a reversible inhibition at 60°, an inhibition which might become non-reversible if we

[7] The sample of acetone at hand was not particularly pure.

kept the pepsin at 60° long enough to test directly the power of digestion. We can, however, heat photogenin and photophelein to 60°, cool them and find out immediately if they give light.

### PROTEIN PRECIPITANTS

The light disappears in a phosphorescent mixture of photogenin and photophelein when the following substances are added to the concentrations indicated: tannin—m/2500 to m/5000; phosphotungstic acid—m/2500 to m/5000; picric acid—m/500, assuming a saturated water solution to be 1.03 per cent or m/22.

A peculiar phenomenon observed with phosphotungstic and tannic acids but not with picric acid is, that, at the lower limit of extinction, the light reappears after first disappearing and more solution must be added in order to extinguish it again. Care was taken to make sure that the phenomenon was not due to more perfect adaptation of the eyes to the dark. When the light is extinguished by dilute picric acid it will reappear again on dilution with water.

### ACIDS AND ALKALIES

The effect of acids (HCl) and alkalies (NaOH) can be seen by adding 1 cc. of the acid (or alkali) of a definite concentration to 1 cc. of a glowing mixture of photogenin and photophelein. The concentration of the acid or alkali in the mixture will thus be one-half of the concentration added. The results are given in table 3.

TABLE 3

*Effect of acids and alkalies on light production*

| CONCENTRATION IN MIXTURE | CHARACTER OF LIGHT |
|---|---|
| n/250 NaOH | Instantly disappears |
| n/500 NaOH | Instantly very dim, lasting > 5 minutes |
| n/1000 NaOH | Instantly dim, lasting > 5 minutes |
| n/2000 NaOH | Instantly fair, lasting > 5 minutes |
| n/4000 NaOH | Instantly fair, lasting > 5 minutes |
| n/8000 NaOH | Instantly fair, lasting > 5 minutes |
| Control | Remains good > 5 minutes |
| n/2000 HCl | Instantly disappears |
| n/4000 HCl | Instantly dim, lasting > 5 minutes |
| n/8000 HCl | Remains good > 5 minutes |

The effect of acid (HCl) and alkali (NaOH) was also studied in the following way: 1 cc. fairly dilute (one *Cypridina* to 10 cc.) photophelein was mixed with 1 cc. n/500 HCl, allowed to stand one hour and then neutralized by adding an equal volume of n/1000 NaOH and tested for light by adding photogenin. The photophelein was therefore in contact with n/1000 HCl for one hour. In this way the effect of acid on photogenin and photophelein can be separated. The results are given in table 4.

TABLE 4

*Effect of acid and alkali on photophelein and photogenin*

| CONCENTRATION OF HCl | EFFECT ON PHOTOPHELEIN NEUTRALIZED AFTER ONE HOUR AND PHOTOGENIN ADDED | EFFECT ON PHOTOGENIN NEUTRALIZED AFTER ONE HOUR AND PHOTOPHELEIN ADDED |
|---|---|---|
| n/1000 | Negative | Negative |
| n/2000 | Very faint | Negative |
| n/4000 | Bright | Very faint |
| n/8000 | Bright | Bright |
| Control in water | Bright | Bright |
| CONCENTRATION OF NaOH | | |
| n/31 | Faint | Negative |
| n/62 | Faint | Negative |
| n/125 | Faint | Negative |
| n/250 | Faint | Faint |
| n/500 | Faint | Fair |
| n/1000 | Faint | Fair |
| Control in water | Good faint | Fair |

Even very small concentrations of NaOH added to a glowing mixture of photogenin and photophelein will decrease greatly the amount of light (table 3). Thus if we add 1 cc. n/500 NaOH to 1 cc. of a brightly glowing mixture, making the concentration of NaOH n/1000, the light instantly becomes very dim and remains so for a considerable time. If NaOH be added to a concentration of n/250, the light disappears completely; if we now neutralize the NaOH with n/250 HCl, the light returns, faint.

Still smaller concentrations of HCl prevent light production. Thus, (table 3) n/2000 HCl extinguishes the light from a luminous mixture, but on neutralization with n/2000 NaOH, the light returns and is fairly bright. The effect of NaOH and HCl is therefore to inhibit light production, and not to immediately destroy the photogenin and photophelein (tables 3 and 4). Note also from table 4 that the photo-

genin is more readily affected by HCl and especially by NaOH than the photophelein.

In the case of acid and alkali as with so many other substances on the light-giving material, the effect is reversible if the substance is removed. The concentration of HCl which inhibits light production is the same as that affecting cells, although the concentration of NaOH necessary to inhibit light production is somewhat greater than that necessary to affect cells (15).

### POTASSIUM CYANIDE

The effect of KCN is of especial interest because of its power of inhibiting cell oxidations. It was tested by mixing an equal volume of photogenin (one *Cypridina* to 25 cc.) with the KCN solution, and testing with an equal volume of photophelein (one *Cypridina* to 12 cc.) after ten minutes and after one hour. Table 5 gives the results.

TABLE 5

*Effect of KCN on light production*

| CONCENTRATION OF KCN IN PHOTOGENIN SOLUTION | LIGHT WITH PHOTOPHELEIN AFTER 10 MINUTES | LIGHT WITH PHOTOPHELEIN AFTER 1 HOUR |
|---|---|---|
| n/20 | Fair | |
| n/30 | Fair | Fair |
| n/125 | Fair | Fair |
| n/250 | Bright | Bright |

In *Cypridina* as in all other luminous animals which I have tried (*Cavernularia* (4), *Noctiluca* (2), fire-fly (3), luminous bacteria),[8] KCN is practically without influence on light production. *Cavernularia* juice, for instance, will light for over 90 minutes in n/40 KCN. On the other hand, n/1280 KCN is sufficient to completely inhibit the oxyluminescence of pyrogallol by the vegetable oxidases (10).

### SATURATION WITH SUGAR, $(NH_4)_2SO_4$ AND NaCl

Saturation of a luminous mixture of photogenin and photophelein with sugar or NaCl causes the light to disappear and it reappears on dilution of the mixture with water.

Since $(NH_4)_2SO_4$ is acid a small amount of this salt causes the light to disappear and it does not return upon dilution.

[8] Unpublished experiments.

Probably these phenomena are connected with the salting out of the luminous substances, although no signs of a precipitate are visible when the natural secretion of *Cypridina* is saturated with NaCl or $(NH_2)_2SO_4$. As already pointed out, this result is no doubt due to the small concentrations of the substances present.

### CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN IN *Cypridina*

In the normal secretion of *Cypridina*, there is more photogenin than photophelein, as may be seen by adding fresh photophelein to the normal secretion after the light has disappeared on standing. The light again appears. The photophelein had been completely used up. This may be shown in another way by allowing a concentrated mixture of photogenin and photophelein to stand until the light disappears, and then boiling one half of the mixture. Upon mixing the two halves no light results, as all the photophelein had been used up before the photogenin was destroyed in the tube boiled.

The concentrations of the two substances which will give visible light when mixed are very small. In one experiment performed 15 minutes[9] after the photophelein was obtained, one *Cypridina* in 25,600 cc. of water gave a just visible light when mixed with an equal volume of more concentrated photogenin. The photogenin from one *Cypridina* in 25,600 cc. water will also just give visible light when mixed with an equal volume of photophelein.

We can see how small an amount of photogenin will give light when we consider that the animal is about 3.5 by 2.5 by 1.5 mm. and that the luminous gland is more or less of a cylinder 0.64 high by 0.24 mm. diameter with a volume of about 0.03 mm. or 0.00003 cm. and that one luminous gland in 25,600 cc. water will give visible light when mixed with an equal volume of photophelein, or in a concentration of 51,200 cc. Hence one part of gland in about 1,700,000,000 parts of water will give visible light upon the addition of photophelein. Even this is a low estimate, as we do not know what the concentration of the photogenin is in the gland itself.

When we compare the amount of photogenin which can be detected by light production with the amount of substances detectible by chemical means we see how extraordinarily sensitive the light reaction is. AgCl is soluble in water to the extent of one part in 625,000 parts

[9] The experiment was performed as quickly as possible because the photophelein disappears on standing.

water at 20° (12) so that more chloride than this must be present to be detected by $AgNO_3$, a very delicate reaction. $KMnO_4$ is roughly just distinguishable by its color in a 14 mm. test tube in a concentration of 1: 1,250,000.

Biological reactions are, generally speaking, more delicate than inorganic reactions. Zinc in traces has a favorable influence on the growth of moulds and 1: 25,000,000 will increase the weight of a crop of *Aspergillus* by 50 per cent (13). Copper will kill *Spirogyra* in one minute when present in 1: 77,000,000 concentration (13). According to Kastle (14), blood in 1: 80,000,000 can be recognized by the phenolphthalin reaction. Since this reaction is due to hemoglobin, of which blood contains about 10 per cent, the hemoglobin can be recognized in 1: 800,000,000 concentration. These figures give an idea of the delicacy of the photogenin-photophelein reaction as compared with others. It should be borne in mind that pyrogallol in 1: 254,000 will give visible light with potato juice and $H_2O_2$ (10).

## IS PHOTOGENIN USED UP IN LIGHT PRODUCTION WITH PHOTOPHELEIN?

The exceedingly small concentration of light substances which give visible light suggests that one or both are of enzyme nature as Dubois supposes. There are two ways of testing this question. One is to determine whether a small concentration of photogenin can use up a large concentration of photophelein providing a sufficiently long time is allowed. We can not use this method because photophelein decomposes spontaneously.

Another way is to determine if a small amount of photogenin can decompose successively added amounts of photophelein without itself undergoing diminution. This method is not unequivocal since many true enzymes are paralyzed or destroyed by the decomposition products of the reaction which they accelerate.

It was found that if we add to 1 cc. of a weak (one *Cypridina* to 50 cc.) solution of photogenin, successive 1 cc. additions of a concentrated (one *Cypridina* to 2 cc.) solution of photophelein as soon as the light from the preceding addition has disappeared, after four 1 cc. additions no more light will appear. The photogenin is therefore used up and can not oxidize additional photophelein, although there is plenty of photophelein present, as can be shown by adding fresh photogenin when a good light appears. With each successive addition of concentrated photophelein, the light which at first is very bright and lasts

about one hour, becomes less brilliant and lasts a shorter time. This is not due to mere dilution of the dilute photogenin as we can dilute the dilute photogenin to the same volume with water and then upon adding photophelein a good light results. We can only conclude that, although photogenin can use up a large amount of photophelein, it is itself changed in some way in the reaction and disappears. We can not say how much photophelein will combine with a definite quantity of photogenin because we do not know the absolute amount of these substances in a single *Cypridina*. In the above experiment we added a concentration of photophelein from one *Cypridina* 100 times (i.e., four additions each 25 times more concentrated), that of the photogenin from one *Cypridina*.

Although the evidence goes to show that the photogenin is used up it is not nearly so rapidly used up as is the oxidase of potato in the production of light from pyrogallol (10). We must remember also that certain enzymes, as zymase, (thermolabile and non-dialyzable) are only active in presence of a co-enzyme which is not destroyed by heat and is easily dialyzable. In fact the photogenin-photophelein system resembles to a very remarkable degree the zymase-co-zymase system. There is the same quantitative relation between zymase and co-zymase as between photogenin and photophelein. If zymase is present in excess, the co-enzyme is all used up; if co-zymase is in excess then the zymase is used up (16). Nevertheless, however much photogenin resembles co-zymase, I have for the present deemed it best to avoid the termination ase. In absence of more definite knowledge we may provisionally regard photogenin as a substance autoöxidizable only in presence of photophelein.

If we try the reverse experiment, that of adding 1 cc. of concentrated (one *Cypridina* to 2 cc.) photogenin to 1 cc. of dilute (one *Cypridina* to 50 cc.) photophelein, a bright light lasting ten to fifteen seconds appears and no more light upon adding additional concentrated photogenin. As might be expected the small amount of photophelein is very rapidly used up by the large amount of photogenin.

The quantitative relations between concentration of photophelein and photogenin, duration of light, and brightness of light, will be considered in a subsequent paper.

## SUMMARY

1. The luminous secretion of *Cypridina* comes from several spindle shaped yellow gland cells on the upper lip and is extruded to the sea water as perfectly clear granule-free non-fluorescent secretion. The light is homogeneous and bluish white in color.

2. If the luminous secretion stands, the light disappears, and if we now add an extract of *Cypridinas* heated to boiling, the light again returns; i.e., *Cypridina* gives a luciferin-luciferase reaction similar to *Pholas dactylus* as described by Dubois.

3. Contrary to Dubois' theory, the luciferase is the source of the light, and not an enzyme causing light production by oxidation of luciferin, because we can obtain light from luciferase by substances incapable of oxidation (NaCl, chloroform, ether, etc.). The new names of photogenin or light producer for luciferase, and photophelein or light assistor for luciferin have therefore been proposed.

4. Oxygen is necessary for light production.

5. Both photogenin and photophelein will pass a Pasteur-Chamberlain or Berkefeld filter easily.

6. Photophelein dialyzes readily through heavy parchment or collodion, photogenin with great difficulty or not at all, even after a period of thirty-six hours.

7. Both photogenin and photophelein are adsorbed by bone-black and $Fe(OH)_3$.

8. The light-producing substances may be dried and thoroughly extracted with ether without impairing their light-giving power.

9. Chemical tests on the natural light secretion give negative results since a very small amount of light substance gives a bright light and at least one part of photogenin or photophelein in 1,700,000,000 parts of water will give a visible light.

10. Photophelein occurs throughout the body of *Cypridina*, photogenin only in the luminous organ. Photophelein from non-luminous parts disappears (apart from photogenin) on standing, but not so readily if the extract has been boiled or in the absence of oxygen. In the fire-fly and some non-luminous beetles, the photophelein disappears so quickly that it is best to make the extract with boiling water. In a non-luminous species of *Cypridina*, a photophelein occurs in small quantity with properties similar to that in *Cypridina hilgendorfii*.

11. Photophelein occurs in extracts (both boiled and unboiled) of many non-luminous organisms, in greatest quantity in *Lepas anati-*

*fera*. The photophelein of *Lepas anatifera* does not disappear on standing.

12. Pure protein solutions (peptone, Na nucleoproteinate, albumen, etc.) or dried mammalian blood do not contain photophelein, but urine contains a similar body.

13. While photogenin will give light with many substances known and unknown, photophelein will give light only with the photogenin of luminous organs.

14. *Cypridina* photogenin will give no brighter light with extracts (photophelein) of other luminous forms (*Luciola*, *Cavernularia*, *Noctiluca*) than with extracts of non-luminous forms, and *Luciola* photogenin will not give so bright a light with *Cypridina* photophelein as with boiled extracts of non-luminous insects. We must conclude that the two substances are not specific for luminous forms, although there is a certain amount of specificity, for photogenin gives the best light with photophelein from the same species.

15. Bright light can be formed by *Cypridina* at 0°C. Photogenin is destroyed above 70°, the temperature and time depending on the concentration; photophelein only after several minutes boiling, the time depending also on the concentration. The natural luminous secretion ceases to light at 52 to 54°, but the light returns on cooling.

16. Photophelein is relatively unstabile and disappears on standing, the time depending on the concentration. Photogenin is much more stabile but also disappears slowly.

17. The spontaneous decomposition of photophelein is retarded (perhaps prevented) by lack of oxygen but hastened by addition of preservatives (chloroform, ether, benzol, thymol). Photogenin can be kept longer by addition of preservatives. One sample preserved with chloroform retained its power to give light with photophelein for over fifty-six days at room temperature.

18. Saturation with ether, chloroform, benzol, thymol or chloretone does not affect the light from a mixture of photogenin and photophelein. Saturation with butyl alcohol or 20 per cent ethyl alcohol or 16 per cent acetone extinguishes the light, and if the mixture is diluted with water, the light reappears. This phenomenon of *anesthesia of a solution* is given by photogenin filtered through porcelain plus photophelein and difficultly soluble butyl alcohol, so that it can not be due to the presence of cell fragments or to insolubility in the 20 per cent ethyl alcohol.

19. Picric acid, tannic acid and phosphotungstic acid extinguish the light in very weak concentrations, and the light returns in the case of

picric acid if the solution is diluted with water. In the case of tannic and phosphotungstic acids, the light returns after first disappearing even *without diluting*.

20. HCl between n/2000 and n/4000 and NaOH between n/250 and n/500 extinguish the light, and the effect is reversible upon neutralization. It can be shown that both HCl and NaOH affect the photogenin more readily than the photophelein and that the effect of NaOH especially is more readily reversible.

21. KCN does not inhibit light production even in strong concentration.

22. Saturation with $(NH_4)_2SO_4$, NaCl or cane sugar extinguishes the light most readily in the order named, and the effect is reversible except in case of $(NH_4)_2SO_4$ (due to acidity).

23. In the natural secretion of *Cypridina* or in the whole animal there is always enough photogenin to completely use up the photophelein. The photogenin from one animal will use up a large additional amount (at least 100 times the concentration in one animal) of photophelein, but not an indefinite amount, so that photogenin is not a true enzyme in the strict sense of the word, unless it be an enzyme poisoned by its own reaction products. The photogenin-photophelein system resembles the zymase-co-zymase system to a remarkable degree, but it is best for the present to regard photogenin not as an enzyme but merely as a substance autoöxidizable only in presence of photophelein.

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# STUDIES ON BIOLUMINESCENCE

## V. The Chemistry of Light Production by the Fire-Fly

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The experiments recorded herein have been made during a period of two years on several different species of fire-fly—the American *Photuris pennsylvanica* and *Photinus pyralis*, the Japanese *Luciola vitticollis* and *parva* and the West Indian "Cucullo," *Pyrophorus noctilucans* and *havaniensis* All the species are essentially similar in behavior, and in general the statements apply to all.

In the second paper of "Studies on Bioluminescence" (1) I pointed out that the fire-fly gave Dubois' (2) luciferin-luciferase reaction (i.e., an extract of the luminous organ allowed to stand until the light disappears will again give light if mixed with an extract of luminous gland which has been boiled), and that luciferin from one species or genus of fire-fly would give light with luciferase from another species and vice versa. In the fourth paper of the series, (3) I showed that in *Cypridina* Dubois' luciferase (the thermolabile substance) was the source of the light and not luciferin (the thermostabile substance) as Dubois' theory supposes, and I proposed that these substances be called photogenin (light producer), equivalent to luciferase, and photophelein (light assistor), equivalent to luciferin. Photogenin (luciferase) will use up a large amount of photophelein (luciferin) but itself also disappears in the process so that it seems best to avoid the enzyme termination *ase* for the present. Photogenin appears to be a substance auto-oxidizable with light production in presence of photophelein.

Since the fire-fly differs from *Pholas* and agrees with *Cypridina* in important points—in the fact that fire-fly photophelein (luciferin) can not be oxidized with oxidizing agents, and is found in many non-luminous forms—I have also applied the names photogenin and photophelein to the light producing substances of the fire-fly.

A brief account of the properties of fire-fly photogenin and photophelein as compared with other luminous forms follows.

## DISTRIBUTION OF PHOTOGENIN AND PHOTOPHELEIN

While photogenin is found only in the luminous gland cells of the fire-fly, photophelein is distributed throughout the body of the fire-fly, the opposite of the condition described by Dubois for *Pholas*. An extract of the non-luminous parts of the fire-fly will give light with photogenin, but only if tested immediately the extract is made. An extract ten minutes old is found to contain no photophelein unless it has been previously boiled. After boiling the photophelein can be kept for many days without decomposition. There appears to be something in the extract of non-luminous parts of the fire-fly which causes the photophelein to disappear unless the former is destroyed by heat. That this is the case can be shown by adding unboiled extract of the non-luminous parts to photophelein, when the latter disappears from solution. Because of failure to boil the non-luminous fire-fly extract, I had previously overlooked the existence of photophelein in regions other than the luminous gland. Lack of oxygen retards (prevents?) the disappearance of photophelein from non-luminous parts, showing that an oxidation is involved. Note that just as photophelein disappears in contact with photogenin and oxygen with light production, so it also disappears in contact with this substance in non-luminous parts, but without light production. In this respect the fire-fly agrees perfectly with *Cypridina*.

Among non-luminous forms, we find some whose extract will give light with photogenin whether boiled or unboiled, others whose boiled extract only will give light, and others whose extract will give no light whether boiled or unboiled. The first mentioned presumably contain photophelein not readily destroyed by standing, the second are similar to the fire-fly and the third may contain no photophelein or some substance very quickly destructive to photophelein or an excess of acid or perhaps merely unstabile photophelein. The exact reasons have not yet been worked out. The following extracts were tried:

Luciola photogenin × caterpillar (sp?) blood.....bright
Luciola photogenin × caterpillar (sp?) blood, boiled.....bright
Luciola photogenin × beetle (coccinella 7-punctata) extract.....negative
Luciola photogenin × beetle (coccinella 7-punctata) extract, boiled.....bright
Luciola photogenin × beetle (Glysiphana jucunda) extract.....negative
Luciola photogenin × beetle (Glysiphana jucunda) extract, boiled.....bright
Luciola photogenin × beetle (Anomala rufscuprea) extract.....negative
Luciola photogenin × beetle (Anamola rufscuprea) extract, boiled.....negative
Luciola photogenin × grasshopper (Pachytylus danicus) extract.....very faint

Luciola photogenin × grasshopper (Pachytylus danicus) extract, boiled....faint
Luciola photogenin × myriapod (Scolopendra sp?) extract.....faint
Luciola photogenin × myriapod (Scolopendra sp?) extract, boiled.....faint
Luciola photogenin × ox blood extract (boiled or unboiled).....negative
Luciola photogenin × neutral potato juice (boiled or unboiled).....negative

Note that the oxidases of blood or potato juice will give no light with photogenin and they also will give no light with photophelein, even if we add $H_2O_2$. As pointed out in my previous papers, although the oxidases can oxidize pyrogallol with light production (4), they have nothing to do with light production by animals.

## CONCENTRATION OF PHOTOGENIN AND PHOTOPHELEIN NECESSARY TO PRODUCE LIGHT

The amount of photogenin in the fire-fly is greater than the amount of photophelein necessary to combine with it; otherwise we would not be able to obtain photogenin because it would be completely used up in combination[1] with photophelein. In some luminous animals (*Noctiluca, Cavernularia, Watasenia*), I have utterly failed to demonstrate a photogenin-photophelein reaction under conditions which should be favorable and after many attempts to demonstrate it. The explanation of this result may lie in the presence of equivalent amounts of the two substances.

Both photogenin and photophelein are found in much smaller concentration in the fire-fly than in *Cypridina*. The photogenin from one fire-fly (*Luciola parva*) whose average size is 8.2 × 3.1 mm. with luminous organ in the ♂ 2.2 × 1.5 mm. and in the ♀ 2.3 × 0.5 mm., will give just visible light in 1.6 cc. water, and the photophelein from one fire-fly in 3.2 cc. water. The greater possible dilution of the photophelein is due no doubt to the fact that the photophelein comes from the whole body while the photogenin is derived only from the luminous gland. When we compare this with the crustacean, *Cypridina*, which will give visible light in 25,600 cc. or one part of luminous gland in 1,700,000,000 parts of water, we see that the fire-fly, despite the brilliancy of its light, is not a very powerful light producer.

[1] I have not actually made the experiment to show that photogenin is used up in giving light with photophelein, but it seems highly probable that this is the case in the fire fly as it is in *Cypridina*.

*Comparative properties of photogenin and photophelein in Cypridina, fire-fly and Pholas*

| PROPERTY | CYPRIDINA (HARVEY, 3) | | FIRE-FLY | | PHOLAS (DUBOIS, 2) | |
|---|---|---|---|---|---|---|
| | Photophelein | Photogenin | Photophelein | Photogenin | Luciferin (Photophelein) | Luciferase (Photogenin) |
| Destruction temperature | Boiling 1–5 minutes | 70° or above. (light disappears 52–56° returns on cooling) | Not destroyed by 10 minutes boiling | 42° | Above 70° | 60° |
| Distribution | Throughout *Cypridina* and many non-luminous forms | Luminous organs of *Cypridina* | Throughout fire-fly and many non-luminous forms | Luminous organs of fire-fly | Luminous organ of *Pholas* | *Pholas* and many non-luminous forms |
| Stability | Less stabile | More stabile | More stabile | Less stabile | More stabile | Less stabile |
| Porcelain filtration | Positive | Positive | Positive | | With difficulty | With difficulty |
| Dialysis | Easily | Very slowly | Easily | Negative (?) | Slow | Negative |
| Adsorption by bone-black and $Fe(OH)_3$ | Positive | Positive | Negative | | | |
| Light with oxidizing agents | Negative | Negative | Negative | Negative | Positive | Negative |
| Light with saponin, thymol, NaCl, etc. | Negative | Positive | Negative | Negative | | |
| Ether solubility | Negative | Negative | Negative | Negative | Negative | Negative |
| Concentration just giving light | 1 : 1,700,000,000 | 1 : 1,700,000,000 | | | | |
| Substance from one animal gives just visible light in | 25,600 cc. | 25,600 cc. | 3.2 cc. for *Luciola parva* | 1.6 cc. for *Luciola parva* | | |

*Comparative properties of photogenin and photophelein in Cypridina, fire-fly and Pholas—Continued*

| PROPERTY | CYPRIDINA (HARVEY, 3) | | FIRE-FLY | | PHOLAS (DUBOIS, 2) | |
|---|---|---|---|---|---|---|
| | Photophelein | Photogenin | Photophelein | Photogenin | Luciferin (Photophelein) | Luciferase (Photogenin) |
| Effect of chloroform, ether, benzol, etc. | Fairly stabile | Stabile | Stabile | Quickly affected | Very slowly affected | Slowly affected |
| Effect of alkalies | Not destroyed by n/30 NaOH in 1 hour | Destroyed by n/125 NaOH in 1 hour | | | Forms insoluble alkali-albumin with $NH_4OH$ | |
| | Reversible extinction of light by n/125 NaOH if neutralized immediately | | | | | |
| Effect of acids | Destroyed by n/1000 HCl in 1 hour | Destroyed by n/2000 HCl in 1 hour | | | Not precipitated by acetic and $H_2CO_3$ except in presence of neutral salts | |
| | Reversible extinction of light by n/2000 HCl if neutralized immediately | | | | | |
| Effect of alcohols and acetone | Reversible extinction of light with 20 per cent alcohol, 16 per cent acetone and saturated butyl alcohol | | | | Precipitated by alcohol at 82° unchanged | |
| Effect of tannic, picric and phosphotungstic acids | Reversible extinction of light in n/500–n/5000 concentration | | | | Precipitated by picric acid unchanged | |

| | | | | |
|---|---|---|---|---|
| Effect of KCN | No effect | No effect | | |
| Effect of NaF | | | | Not destroyed by 1:100 |
| Effect of enzymes | | | | Destroyed by trypsin |
| Saturation with $(NH_4)_2SO_4$, NaCl, $MgSO_4$, sugar and glycerine | Reversible extinction except in case of acid $(NH_4)_2SO_4$ | | Precipitated by $(NH_4)_2SO_4$, not by NaCl or $MgSO_4$, Insoluble glycerine | Suspend activity and returns on dilution |
| Color of light | Bluish white | Yellowish white | Greenish blue | |

## PROPERTIES OF PHOTOGENIN AND PHOTOPHELEIN

As to the chemical nature of photogenin and photophelein, nothing definite is known. The photophelein is much the more stable substance and can be preserved until attacked by bacteria or with chloroform, for over 70 days. It dialyses readily through collodion, is not adsorbed by lamp-black, and is not readily affected by ether and benzol. Photogenin, on the other hand, disappears in less than five hours at 25°C., is quickly destroyed by ether, benzol and chloroform, and will not dialyse readily if at all.

In this ready destruction of photogenin by the fat solvent anaesthetics, the fire-fly resembles *Pholas dactylus* and differs markedly from *Cypridina*. One sample of *Cypridina* photogenin was preserved under chloroform for over 54 days and still gave light with photophelein.

*Luciola* photogenin is destroyed at about 42°, while the photophelein is still active after ten minutes boiling. A bright light is produced on mixing the two at 0°C.

Very weak concentrations of acids prevent the production of light of the fire-fly, less weak concentrations of alkalis are necessary and KCN does not affect light production in strong (m/100) concentrations.

The following table summarizes the characters of photogenin and photophelein (luciferase and luciferin) as found by Dubois for *Pholas* and by myself for *Cypridina* and the fire-fly. Where a statement runs through both photogenin and photophelein column it applies to a mixture of the two substances. A blank indicates that the experiment has not been tried.

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# STUDIES ON BIOLUMINESCENCE

## VI. Light Production by a Japanese Pennatulid, Cavernularia haberi

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Panceri (1), working with *Pennatula phosphorea*, has given us the best account of phosphorescence among the pennatulids. He describes the light as coming from eight bands of cells on the outer wall of the stomach and continued into buccal papillae. They contain a luminous "fatty" matter which can easily be squeezed out as a sort of slime and which does not decompose readily. He also found that on stimulation a wave of light would pass over the colony in any direction at a rate of about 5 cm. per second. In a form like *Cavernularia* he describes also somewhat similar conditions (1, p. 40), but his descriptions are very meager. I have found the Japanese species of *Cavernularia* more favorable for light experimentation than *Pennatula* and the following pages contain confirmation of Panceri's experiments on the light waves passing over the colony and a general account of the chemistry of light production.

### GENERAL CHARACTERS

*Cavernularia haberi* is especially abundant in the fjord of Aburatsubo, Japan, near Misaki, the Marine Station of the Imperial University of Tokio. The colony of animals lies hidden in the sand and contracted during the day, but at night takes up water and expands, large ones to the length of 2 feet. If stimulated by touching or electrically or by the addition of ammonia, a slime is formed similar to that produced by most of the *Cnidaria* upon irritation, but differing in that it is brightly luminous. The whole of the outer surface of the colony can form the luminous slime, but not the spongy inner material. The stalk, containing no polyps, is especially brilliant.

The slime may be dried over $CaCl_2$ and will give light when moistened with sea water or fresh water.

By squeezing *Cavernularia*, from which most of the sea water has been gently pressed, one can easily obtain a luminous juice which is still luminous when filtered through filter paper and retains its luminescence for several hours. Examination of the filtrate under the microscope in the dark shows that the light comes from minute points of light which makes the field of view look like the starry heavens. If water is added to this sea water juice, the light is greatly increased due to the appearance of numerous additional points of light. These points of light come from minute granules and globules easily visible in the filtered juice under the microscope. The addition of water to a dark *Cavernularia* juice which has stood for two days will cause the appearance of light due to the dissolving of the granules and globules. So sensitive are they to the addition of water that one drop of fresh water added to 5 cc. of juice will produce light, and so bright is the light that the addition of one drop of *Cavernularia* juice to 5 cc. of fresh water produces a light easily visible when the drop is mixed with the 5 cc.

By centrifuging the filtered juice, the granules may be partially thrown down and will give a brilliant light if fresh water is added while the liquid itself (turbid in appearance) gives a much fainter light. No light is produced upon the addition of water to a parchment paper sea water dialysate of the juice, so that light production is undoubtedly connected with the visible globules and granules of the juice. That it is connected with the solution of the granules is indicated by the fact that fresh water but not salt water or cane sugar is able to call forth the production of light. The process appears to be similar to the cytolysis of cells as can be observed by an inspection of table 2 which shows the effect of adding various substances to the *dark* juice of *Cavernularia*.

The light giving granules of *Cavernularia* will pass through an alundum filter crucible (R A 84) of the finest pores but not through a Pasteur-Chamberlain filter tube. The liquid passing through the latter is perfectly clear and non-luminous and gives no light when water is added.

There is no adsorption of the light producing substance by bone black or $Fe(OH)_3$.

## ELECTRICAL STIMULATION

The juice of *Cavernularia* filtered through filter paper does not respond to the strongest interrupted induced shocks. The living colony, however, responds readily.

When a galvanic current is passed through one of the excised polyps mounted between non-polarizable electrodes, a flash of light occurs on the make and a series of flashes while the current is passing, which cease on the break. There is no flash of light on the break. A similar response can be observed with *Noctiluca* (2). It will be remembered that Romanes (3) observed a series of contractions in the bell of a medusa during the passage of a galvanic current, and the sartorius muscle of the frog often contracts on the make of a galvanic current, remains contracted during the passage of the current and relaxes on the break.

If the whole colony be stimulated by weak induced shocks, there is a local production of light. There is usually no response to a single shock, but a ready response to three or more sent in in rapid succession. With stronger stimuli, a wave of light, easily followed by the eye, passes over the colony in each direction from the point stimulated. With interrupted induced shocks, a series of waves of light follow one another in quick succession (not corresponding to the number of stimuli, however), reminding one of the series of electric shocks given out by the torpedo, only on a slower scale. The time interval between separate flashes no doubt corresponds to the refractory period of the cells concerned.

On pressing deeply into the tissue and stimulating strongly, a much brighter light response also results which very slowly moves away from the point of stimulation and usually does not extend more than 2 or 3 cm. At the same time the whole colony contracts, the polyps are drawn in and in this condition do not respond to electrical stimulation by light production.

The wave of light above mentioned will pass in any direction over the colony and across a cut around the middle of the colony, involving the whole of the external tissue. Some inner tissue must therefore be capable of conducting the stimulus.

## LACK OF OXYGEN

That the production of light by *Cavernularia* is an oxidation can be very readily determined by passing a current of hydrogen through the juice, when the light disappears but promptly reappears upon admitting

oxygen. A sample of phosphorescent juice kept twenty-four hours in an atmosphere of hydrogen gave light when air was admitted.

Considerable oxygen is necessary as may be seen by keeping the juice in a tall test tube, when it lights only at the surface in contact with air. The light appears throughout the tube, however, if mixed with air.

### REDUCTASE, OXIDASE AND CATALASE

A tube of *Cavernularia* juice shut off from the air very quickly reduces (decolorizes) methylene blue. The blue color reappears when oxygen is admitted. This reaction has nothing to do with light production, however, as it is given also by a non-luminous sea anemone (Anthopleura xanthogrammica) and many other animal tissues which can not produce light.

Unboiled *Cavernularia* juice also gives oxidase reactions upon addition of $H_2O_2$ with guaiac, pyrogallol (slight), a-napthol, para-phenylen diamine, indophenol reagent, ortol and pyrocatechin. The boiled juice gave a slight positive test with guaiac, para-phenylen diamine and the indo-phenol reagent. Many other non-luminous tissues give these reactions, however, and there is abundant evidence to show that light production in animals is not connected with the ordinary oxidases (peroxidases) (4).

As in all other organic tissues, catalase is also present in the juice of *Cavernularia*.

### TEMPERATURE

The juice of *Cavernularia* still gives a good light at 0°C., as do pieces of the colony if stimulated.

On raising the temperature, the light of *Cavernularia* juice disappears at 52° and does not reappear on cooling. Pieces of the colony slowly heated begin to light spontaneously at about 40°.

The light of *Noctiluca* (2) disappears at 48°, *Cypridina* (5) at about 54°, a hydroid, *Sertularia* sp. at 54°, the fire-fly, *Luciola* (6) at 42°, and luminous bacteria at 38° (8).

### PHOTOGENIN AND PHOTOPHELEIN

Unlike the fire-fly, *Cypridina* and *Pholas dactylus*, it is impossible to separate the luminous juice of *Cavernularia* into two substances, photogenin and photophelein (luciferase and luciferin) (6), one destroyed by boiling, the other not, which will give light when mixed

(4, 5). We can not, for instance, cause light to appear in *Cavernularia* juice which has stood until the light has disappeared by adding fresh luminous *Cavernularia* juice heated to boiling and then cooled; neither can we obtain light by adding juice heated to temperatures below boiling (88°, 81°, 71°, 61° or 52°C.) and then cooled.

Another species of Pennatulid, *Pennatula* sp., as also *Noctiluca* and the squid, *Watasenia scintillans* behaves as does *Cavernularia*. None of these organisms gives the photophelein-photogenin (luciferin-luciferase) reaction for reasons at which we can only guess. The photogenin or photophelein may be, either of them, very unstabile or there may be sufficient photophelein to use up all the photogenin. The evidence in this case seems to indicate that the photophelein is unstabile as we can obtain a faint light with *Cypridina* photophelein or fire-fly photophelein (both prepared with sea water) and the non-luminous *Cavernularia* juice (photogenin). *Pennatula* and *Noctiluca* photophelein gave negative results with *Cavernularia* juice and vice versa.

The question may be asked—what substances are able to cause light to appear in the juice of *Cavernularia* which has stood until completely dark? Usually four to six hours are sufficient time for the juice to lose its luminescence. It is still capable of giving out a bright light if we add fresh water (but not sea water) to it, and it retains this potentiality for over two days at 20°C. (see table 1) and a shorter time at higher temperatures. As only fresh water and not sea water will call forth the light, and as we know that light production is connected with the granules of the juice, the process appears similar to the cytolysis of cells—i.e., to the swelling and solution of the granules which the cells contain, in fresh water.

By this means something is liberated from the granules of *Cavernularia* juice which oxidizes with light production. Table 2 gives the results of adding various other substances, pure or dissolved in sea water, to the dark *Cavernularia* juice. Note from the table that

TABLE 1

*Effect of standing on light producing power of Cavernularia juice*

| | |
|---|---|
| Juice + water | Bright light |
| Juice 12 hours old at 20°C. + water | Bright light |
| Juice 24 hours old at 20°C. + water | Fair light |
| Juice 48 hours old at 20°C. + water | Faint light |
| Juice 72 hours old at 20°C. + water | None |

TABLE 2

*Effect of substances in calling forth light from non-luminous cavernularia juice*

| SOLUTION OR SUBSTANCE ADDED | CHARACTER OF LIGHT |
|---|---|
| 50 per cent sea water | Bright light |
| 66 per cent sea water | Fair light |
| 75 per cent sea water | Fainter light |
| 80 per cent sea water | Very faint light |
| 90 per cent sea water | None |
| Water | Bright light |
| Sea water | None |
| Sea water evaporated to one-half volume | None |
| m cane sugar | None |
| m/2 cane sugar | Faint light |
| m/4 cane sugar | Fair light |
| m/8 cane sugar | Good light |
| Chloroform saturated sea water | Faint light |
| One-half Chloroform saturated sea water | Very faint light |
| One-quarter Chloroform saturated sea water | None |
| Chloretone saturated sea water | None |
| One-half Chloretone saturated sea water | None |
| One-quarter Chloretone saturated sea water | None |
| Thymol saturated sea water | Fair light |
| One-half Thymol saturated sea water | Faint light |
| One-quarter Thymol saturated sea water | Very faint light |
| Benzol | Faint light |
| Ether | None |
| Chloroform | None |
| Chloretone crystals | Very faint light |
| Thymol crystals | Faint light |
| Chloral hydrate crystals | Faint light |
| Saponin powder | Fair light |
| Oleic acid | Faint light |
| Ortol crystals | Faint light |
| Hydrochinon crystals | None |
| Resorcin crystals | None |
| Pyrocatechin crystals | None |
| Pyrogallol crystals | None |
| $Ba_2O_2$ powder | None |
| $Na_2O_2$ powder | None |
| $KMnO_4$ crystals | None |
| $K_2Cr_2O_7$ crystals | None |
| $FeCl_3$ | None |
| $FeSO_4$ crystals | None |
| $K_4Fe(CN)_6$ | Faint |
| *Laonome japonica* (an annelid) blood | None |
| Dried ox blood extract in sea water | None |
| *Lepas anatifera* extract | None |

TABLE 2—Continued.

| SOLUTION OR SUBSTANCE ADDED | CHARACTER OF LIGHT |
|---|---|
| *Lepas anatifera* extract boiled | None |
| *Chiton* sp: extract | None |
| *Chiton* sp: extract boiled | None |
| *Onchidium* sp. extract (a pulmonate mollusc) | None |
| *Onchidium* sp. extract boiled | None |
| *Dolabella* sp. blood (a nudibranch) | Faint |
| *Dolabella* sp. blood boiled | None |
| *Sepia esculenta* blood | None |
| *Sepia esculenta* blood boiled | None |
| *Panulirus japonica* blood | Very faint light |
| *Panulirus japonica* blood boiled | None |
| *Cypridina hilgendorfii* photophelein in sea water | Fair light |
| *Luciola vitticollis* photophelein in sea water | Fair light |

light production is not due to dilution of the salts of sea water by adding fresh water, since m cane sugar does not call forth the light. Note also that many cytolytic substances (chloroform, benzol, thymol, etc.) give light, but not the oxidizing agents—$Na_2O$, $KMnO_4$, etc. The blood of certain invertebrates also causes very faint light production, but we can not be sure that this is not due to the fact that the blood is somewhat less concentrated than sea water, although the determinations of other closely allied forms show the salt content to be the same as the sea water in which they live.

## CHEMICAL REACTIONS

The luminous juice of *Cavernularia* contains the luminous substance but mixed with many other substances so that mere chemical tests on the juice are of no value in determining the chemical nature of the luminous substance. The luminous material is salted out along with the other proteins but does not retain its power to phosphoresce long enough to be manipulated by the ordinary chemical methods. The following paragraphs give the results of precipitation by $(NH_4)_2SO_4$, picric acid, alcohol, etc.

If to the fresh filtered luminous juice of *Cavernularia* we add sugar, NaCl, $MgSO_4$, $(NH_4)_2SO_4$ to saturation, or 5 volumes of glycerine, the light disappears. A heavy precipitate forms in $(NH_4)_2SO_4$), a small precipitate in $MgSO_4$ and practically none in NaCl. The precipitates are soluble in sea water. No precipitate is formed in sugar or glycerine. If poured into fresh water or sea water immediately after the light has

disappeared, light is produced by all solutions. If the tubes are allowed to stand for a day, no light is produced by any tube on pouring into fresh water or sea water.

If alcohol or acetone (3 vols.) is added to the juice, a precipitate forms and the light disappears. The precipitate is insoluble and gives no light in sea water or fresh water.

The alkaloidal reagents, picric acid, phosphotungstic acid and tannic acid immediately cause precipitation with disappearance of light. The precipitates are insoluble in sea water or fresh water and produce no light.

Normal acetic acid or n $NH_4OH$, added drop by drop produce no precipitate although the light disappears in weak concentrations.

### ANESTHESIA

Ether, chloroform and benzol cause a fairly rapid disappearance of the light in the order named, the ether most rapid. In turpentine, chloral hydrate, thymol and chloretone, the light stays for a long time and then disappears. There is no reappearance of light from any of the tubes if poured into fresh water or sea water unless we do so immediately the light has disappeared, and then the light is very faint and momentary.

In other words, the juice can not be anesthetized. Even in concentrations of alcohol, ether, chloroform or butyl alcohol which cause a slow disappearance of light, there is no recovery or only a momentary faint glow upon dilution of the solutions with sea water. The alcohols give similar results. About 16 per cent ethyl alcohol will cause the light to disappear in one minute, but there is no recovery or only a very faint momentary glow on diluting with sea water. Saturation with butyl alcohol gives a similar result.

Since reversibility is the *sine qua non* of anesthesia, we must conclude that this juice can not be anesthetized, in this respect differing markedly from the granule free *Cypridina* luminous secretion which can be anesthetized by the alcohols. Also many luminous organisms, for instance luminous bacteria (9), can be truly anesthetized.

### POTASSIUM CYANIDE

Potassium cyanide has no inhibiting effect upon the light production of *Cavernularia* juice. Even in m/40 concentration, the light is still bright after ninety minutes, a result agreeing with all my other experiments on luminous organisms (bacteria, fire-fly, *Cypridina*, *Noctiluca*).

SUMMARY

1. The light of *Cavernularia* comes from a luminous slime, a secretion of gland cells over the outer surface of the colony.

2. The secretion contains small granules which can be seen to emit the light. On standing the light from a sea water extract of *Cavernularia* slowly disappears and will not return on shaking, but reappears if fresh water is added to the juice.

3. The light substance (i.e., the granules) will pass an alundum filter (R A 84)' but not a Chamberlain filter. It is not adsorbed by bone black or $Fe(OH)_3$ and will not dialyse.

4. The light producing granules do not respond to electrical stimulation but the colony gives light with galvanic or induced currents.

5. With galvanic currents a flash appears on the make and a series of flashes while the current is passing which cease on the break.

6. Upon stronger stimulation (with induced currents) a wave of light passes over the colony in all directions from the point stimulated. This wave will pass through the deeper tissues when all the ectodermal tissues are cut.

7. A considerable amount of oxygen is used up in light production and no light appears in its absence.

8. *Cavernularia* juice will decolorize (reduce) methylene blue in absence of oxygen and it also contains peroxidases and catalase.

9. The light is still bright at 0°C. Fragments of *Cavernularia* give off light spontaneously at 40° which disappears at 52°C. and does not return on cooling.

10. The photogenin-photophelein reaction is not given by *Cavernularia*, but a faint light can be obtained with a non-luminous *Cavernularia* juice (photogenin) and *Cypridina* or fire-fly photophelein.

11. The production of light by the granules appears similar to the cytolysis of cells as it occurs with water (but not isotonic cane sugar) and certain cytolytic substances (saponin, chloroform, benzol, oleic acid).

12. The light producing substances are salted out along with the proteins but are not stabile enough for chemical manipulation.

13. The light producing granules can not be anesthetized.

14. Potassium cyanide has no effect on light production.

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